# UNIVERSITY OF TORONTO MEDICAL JOURNAL

## RADIOBIOLOGY

Ontario Cancer Institute

VOLUME XXXVIII

NUMBER 6,

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THE COVER

From the painting by Leon Lhermitte, our cover shows a mid-nineteenth century laboratory. Claude Bernard (1813 - 1878) demonstrates his findings in physiological chemistry using an animal subject and equipment developed in the physical sciences. Student and successor of Magendie in Paris, Bernard was the outstanding leader of his time in physiology. His analysis of the digestive processes is famous; he demonstrated the storage of sugar in liver and muscle; he produced an artificial diabetes with a puncture of the medulla oblongata; he studied the influence exerted by the nervous system upon other organs; investigations of blood conditions led him to study various poisons and their effects hence beginning the study of experimental pharmacology. These are only a few of the things he did.

This issue four of the research scientists from the Ontario Cancer Institute have written excellent articles on the many aspects of Radiobiology. The Journal is indebted to them and to Dr. Arthur Ham for the time and effort given to this edition. We express appreciation and thanks for the favor accorded us in having such fine reviews in our Journal.

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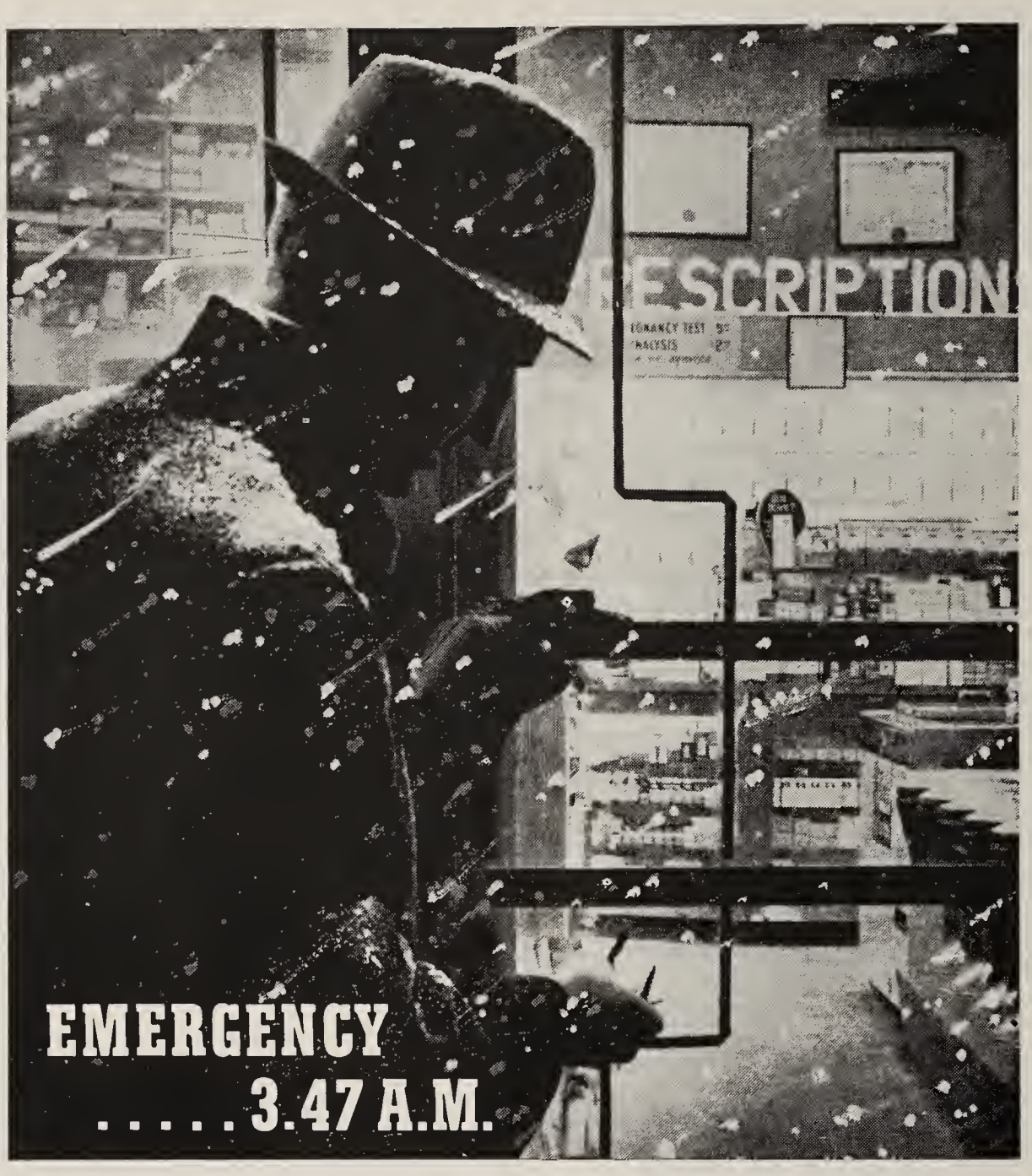


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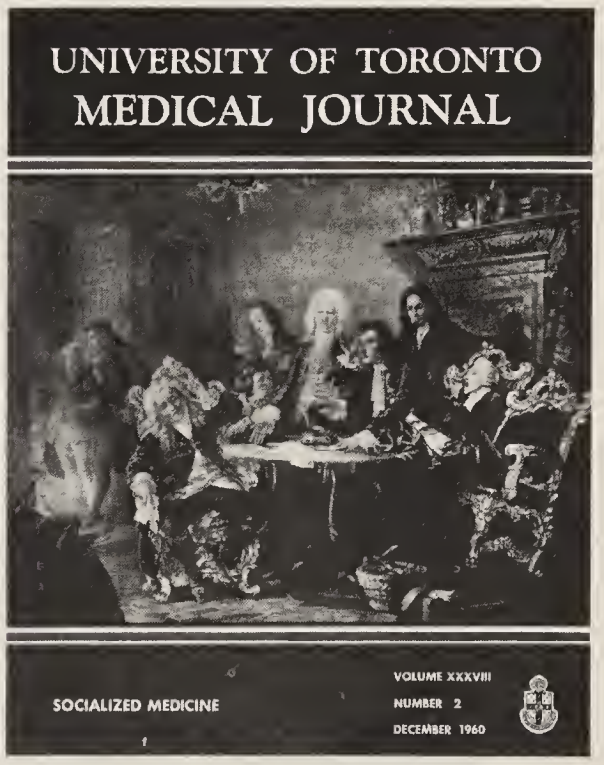

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**Bibliography** (1) Chao, D. H.: *M. Clin. North America* 42:461, 1958. (2) Davidson, D. T., Jr., in Conn, H. F.: Current Therapy 1959, Philadelphia, W. B. Saunders Company, 1959, pp. 509-514. (3) Lemere, F. L.: *Northwest Med.* 53:482, 1954. (4) Perlstein, M. A.: *Pediat. Clin. North America* 4:1079, 1957. (5) Zimmerman, F. T., & Burgemeister, B. B.: *Neurology* 8:769, 1958.

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# EDITORIAL

RETROSPECT

Each year in the final issue, the editor-in-chief usually pronounces a benediction to the poor support of the advertisers and subscribers, takes a shot at medical students about something called apathy, predicts the forthcoming extinction of the Journal, and disgustedly passes the reins to his successor. This year, the editor's hostility was expelled early in the season and the timing of the rage proved effective, for words were transposed into actions which resulted in many successes for the staff of Volume 38.

The old maxim, *"Bull ...t baffles brains"*, toppled the barrier of apathy in the school. As the lone staffer from the previous year, this editor was desperate for help and, consequently, created many fantasies in the minds of prospective enthusiasts. Whatever the truth as they now know it, it can be safely spoken that none of the staff regrets "his" decision to take on the harness of this extracurricular activity without equal. We are very proud of the Journal's thirty-eighth year; I am proud of the staff I picked. Their pictures are found on the masthead page this issue; their names are too numerous to mention here in special thanks but the subscriber knows what they did and this speaks praise in itself.

We were able to finance the Journal without debt, perhaps an historical first. New subscriptions were sold for the first time in many years. With these things plus reorganization of the business aspects, the future for the Journal is bright. There will be more time next year to spend improving the quality of the articles and Journal promotion. More important, there will be staff to carry out these aims; the Journal has always had aims, only rarely has it had men to achieve them. In fact some of the planning for Volume 39 has already been done.

We have received many requests to work with us on the Journal next year from those apathetic medical students. To those who want to ride the bandwagon, we warn you not to even start unless you are sincerely interested in doing *just anything* so long as it is for the good of the book. There is no room for one who fancies himself a writer but not an advertising salesman This is a reasonable demand, for to obtain the money to meet a budget twice that of The Medical Society requires no small amount of work and without this there is no Journal.

There is no larger professional group of semi-literate people than medical doctors. This was confirmed by a former editor of the *Canadian Medical Association Journal.* Student articles edited this year assure me that this state will prevail in future years. How can there be an excuse for simple spelling errors by the dozen made by a University student? Why do medical students insist on writing an article as though it were a cram summary with numbers for each line and one-word sentences? Why do others think that summaries of their lecture notes and a chapter of Harrison constitute a review of the literature? My frustration was epitomized when, after 10 hours spent editing an article written one word under another, with appropriate numbering, the "author" had the stupidity to say, on seeing his article in print, "You didn't change it much, did you?". I began to see that there is more ignorance than carelessness involved in this sorry state. However, this is what the Journal is for and every medical student has the opportunity to improve his writing before it is embarassing him as a graduate. To write an article for a journal one would do well to work on producing a journal first so that he can begin to understand what is required. If a graduate journal receives an ill-put, poorly typed, dry review from you they will put it in the return mail. They will not spend the time to show you what you should know and you may very well never be able to publish anything.

You do not have the inate ability to write. As you have learned to talk so you must learn to write. Never hand an article to an editor with the demand, "I don't want anything edited in this"! Such lack of humility was shown to me twice this year; they were both *lousy* articles. The best way to learn to express yourself on paper is to edit the attempts of others. At least you will never make the intolerable mistakes they do and this alone will most likely catapult your own efforts into the best journals of the country. Although no bard of letters myself I have gained a knowledge of the rudiments which will help the harried editor of any journal. These suggestions appear below.

The Journal, like Pepys, is ready for bed. Next fall it shall awake in the arms of Walter Spitzer, the new editor-in-chief. I fondly hope he enjoys her as much as I did.

L. E. S.

## BASIC STEPS IN WRITING REVIEW ARTICLES

The first thing to ask yourself is, "Do I really want to write this thing?". If you are not sure of the answer to this question then you are better to leave it undone for you will probably do a poor job, this fact leading to the second point. When you are compiling and composing your article keep in mind that you would like to read it ten years from now and still be proud of it. Anything you publish is held for and against you by those who read it so that it is wise to do the best you can. Having committed yourself to some hard work instead of blasphemy, read the editorial requirements of the Journal in which you wish to publish and where necessary consult its editor for clarification.

In the *preparation* of a review article there are a few simple steps to be taken in an organized fashion, else you chase geese. As a student you are well-advised to seek the aid and suggestions of a capable staffman whose speciality is akin to your subject. Here you will find direction for your reading. You will not have to plow through a lot of outdated literature and you will have greater assurance that your own article will be up-to-date if you do this. You next go to Index Medicus and other cumulative indices found in the journal reading room of the Library. Your reading should be organized and thorough, making notes as you go. Be sure to write down all your references at this point rather than search for them after you have completed your effort. At a point which only you can determine an outline should be drawn up to ensure structural integrity. A good review article begins with an historical pearl, a definition and develops the important information in chronological order. Scientific writing must be descriptive and explanatory without being biased in any one direction in the field. It does not of necessity have to be dull reading and there is room for "style" within the boundaries of accuracy.

From your outline a *rough draft* of the final copy should be made and this returned to your mentor, the staffman. A literary friend, if you have one, can also be used to criticize sentence structure and punctuation, etc., and will also ensure that there are no obscure parts. Corrections should be made in the rough draft before final typing.

When making your manuscript use only three types of *headings* (Heading, Subheading and Sub-Subheading). There is no need to number them. Do not break your text down any more than this and use headings only where applicable; do not show your weaknesses by resorting to them. The position of your illustrations must be clearly shown in the manuscript. *Illustrations* must be properly numbered and labelled. The text for all of them is placed on a separate sheet of paper with corresponding numbers. Line drawings must be expertly done in India ink on white paper and should be kept in a separate envelope buttressed by two sheets of stiff cardboard. The same applies to photographs. *Acknowledgments* should be brief and not sicken-

ing. Your summary must briefly state the main points you have made in their proper order. It is this editor's opinion that summaries are a waste of time in a review article but of immense value in an original one. You might consider this and forego any summary. Lastly, your *references* must be in order of appearance in the text. It is always best to place numbers of references in the text at the appropriate positions. The list of references must be uniform and done in the following approved manner: *1. Author's name, Title of Article; Name of Journal, Vol. number, month, year.*

The final stage of preparation is in the *typing*. This Journal requires all articles to be typed (totally unacceptable otherwise) double spaced (triple on some typewriters) with 66 strokes to the line and roughly 25 lines to a standard 8½" x 11" page. For your own benefit make a duplicate. There must be a good margin on either side for editing purposes; following the above pattern this margin will result. Such simple things as these enable the editor to calculate how much final space your article will take, this being of great importance in the overall make-up.

The above rudiments alone will not make your article a success but, if observed, they will carry it further in the editor's consideration. The prime factor in writing is *experience* and if this is based on good habits your path is short-cut. The purpose of a student journal, and definitely this one, is to provide an opportunity for you to gain this experience.

L.E.S.

---

## LETTERS TO THE EDITOR

*Ed. — The following letter is in reference to Associate Editor J.R. Allen's article which appeared in the first issue of Volume 38.*

• *Dear Mr. Allen,* Hearty congratulations on your superb paper on Antigens and Antibodies. I only wish that I could have read it before the page proofs of a new chapter on Immunity and Hypersensitivity for the revised edition of my Textbook of Pathology came in some weeks ago. Then I might have merited the fine write-up of Anderson's Synopsis by Mr. Soper which I have just sent to Doug. Anderson.

The whole journal is just first rate.

Sincerely yours,

William Boyd.
Professor Emeritus of Pathology,
University of Toronto.

• *Dec. 20, 1960.* I am writing to congratulate you and your staff on the new format of the University of Toronto Medical Journal, the last issue of which I received a few days ago. It now looks much more like a professional journal and I have heard nothing but complimentary remarks about the changes on all sides.

Sincerely yours,

James A. Dauphinee.
Professor of Pathological Chemistry,
University of Toronto.

• Kindly forward 100 copies of the February 1961 issue of your Journal to my office. It is my wish to have a copy in the hands of every D.O. in Canada.

Unbiased publications of this nature will go far in formulating a better understanding among those in the healing arts; consequently, the Public Health must ultimately be the one to benefit.

On behalf of myself, my Committee and the D.O.'s in general, I wish you the success which goes with broad-mindedness, tolerance and good judgment.

Very truly yours,

G.R. Church, D.O.
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# RADIOBIOLOGY—An Introductory Survey

J. E. TILL, Ph. D.

One of the effects of recent progress in biological and medical science is that of breaking down the traditional boundaries that formerly segregated individual disciplines. No better example of forces that have operated in this connection can be found than those that have been associated with the development of modern radiobiology. At its present stage of development, radiobiology is more a set of problems than a discipline and these problems impinge on many disciplines. There are few individuals who are exclusively radiobiologists, but there are many physicists and biophysicists, chemists and biochemists, physiologists, pathologists, geneticists, cytologists, microbiologists and other physical, biological and medical scientists who are interested in the problems of radiobiology pertinent to their own particular fields. This fragmentation of radiobiological research among many specialists in part explains why radiobiology is seldom taught as a separate subject in medical school and elsewhere, and more often is introduced in bits and pieces into different courses. Under these conditions, what might be said to be the subject matter of radiobiology is not as yet well organized, since it is often difficult for a specialist in one field to relate his own work to that of specialists in other fields.

Occasionally, a bold individual has tried to create some order out of this chaos. The best attempt of this sort by a single individual was a book by Lea (1), a physicist, the first edition of which appeared in 1947. More often, source books in radiobiology are written co-operatively by groups of specialists. Under these circumstances it is difficult to achieve continuity between separate contributions, and lines of reasoning may occasionally be lost in a welter of detail. The most authoritative series of this type is the three volumes edited by Hollaender (2), published in 1954. The rapid advance of knowledge in recent years however, has rendered both of the above-mentioned works obsolete and no adequate replacement for them exists. In the following pages, a small part of the recent work will be outlined and an attempt will be made to show how the studies in separated fields may be related. Five main areas of investigation will be considered.

I. Radiation physics.
II. Radiation chemistry.
III. Molecular biology.
IV. Cellular biology.
V. Histology and pathology.

In this introductory survey, each of these fields will be considered only in very general terms. A more detailed discussion of some particular aspects of radiation chemistry and molecular biology will be found in Dr. Hunt's paper. Dr. Whitmore's paper covers cellular radiobiology in more detail, and Dr. Bruce's paper gives a much more thorough outline of the histological and pathological aspects of radiation action.

## I. RADIATION PHYSICS

### *Classification of Radiation*

The physical aspects of the interaction of radiation with matter have been rather thoroughly studied and are reasonably well understood. There are various ways of classifying radiations, but for radiobiological purposes, the following classification can be considered:

*Non-ionizing radiations*
Ultraviolet (UV) light.
*Ionizing radiations*
a. Electromagnetic radiations (x- and gamma-rays)
b. Charged particles (electrons, protons, alpha-particles, etc.)
c. Neutrons.

The ionizing radiations are of major interest, and x- and gamma-rays are of the greatest importance.

### *The Absorption of X-rays*

X-rays are very penetrating; when they strike a material, many of them pass right through it without being affected. Some of the rays, however, are absorbed, and it is because of this that it is possible to take an x-ray picture. Such pictures are usually taken with relatively low energy x-rays, because these are very strongly absorbed by dense materials, while they pass relatively easily through less dense materials (3). Thus, bone strongly absorbs low energy x-rays, while soft tissue is relatively transparent, and a good picture can be obtained. High energy x-rays are only weakly absorbed even by dense materials, so that both soft tissue and bone are relatively transparent. This means that an x-ray

film obtained using high energy x-rays shows little contrast between bone and soft tissue, and a poor picture results, as shown in Figure 1.

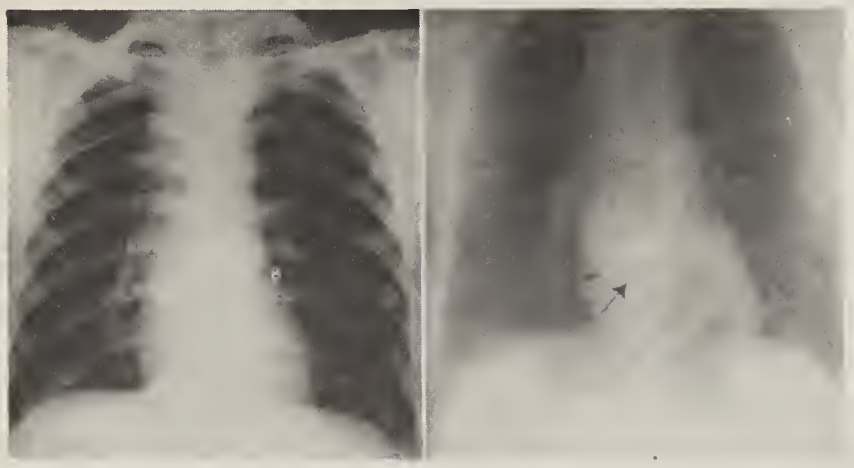

Figure 1. Left: An x-ray film taken with low energy x-rays. Right: A film taken with high energy radiation.

*The Production of Ionizations in Material*

The absorption of x-rays results from an interaction of the radiation with matter. In the interaction some of the energy of the x-ray beam is given over to the absorbing material, serving to ionize or excite atoms in the material. This is an important point which must be realized about the interaction of ionizing radiations with matter. The main effect of exposing matter to ionizing radiation *of any kind* is the production of *ionized atoms in the material,* hence the term "ionizing radiations". There is no reason to expect x-rays, beta-rays, neutrons, or any other ionizing radiation, to have any fundamentally different effects. All act mainly by producing ionizations in the material.

*The Roentgen and the Rad*

At present, the measurement of quantity of ionizing radiation involves one of two units, the *roentgen* and the *rad.* The first is based on the ability of ionizing radiations to produce ionizations in any material, including air. The roentgen is that amount of radiation required to produce a certain number of ionizations (enough to make up one electrostatic unit of charge of either sign, plus or minus) in 0.001293 grams of air (which is one cubic centimeter of air at normal temperature and pressure) (4). The roentgen (abbreviated "r") is used to measure the amount of radiation *produced* by an x-ray machine. The rad, on the other hand, is used to measure the amount of radiation energy *absorbed* by a material being irradiated, and is equal to 100 ergs of energy absorbed per gram of material (4). After exposure of a material to a certain number of roentgens of radiation, the amount of energy absorbed by the material, measured in rads, will depend on the nature of the material, since the interaction of radiation with a material depends on the nature of the material. The roentgen and the rad are not simply related, except for air. The rad is the more meaningful unit, but the roentgen is more easily measured. This has created a dilemma as to which should be used to describe the radiation dose received by a material and at present both are used. For soft tissue being irradiated with high energy x-rays, the so-called "absorbed dose" received by the soft tissue, measured in rads, is different in magnitude by less than 5 percent from the "exposure dose", measured in roentgens, so in this case the distinction is not too important in practice.

## II. RADIATION CHEMISTRY

*Toxic Substances in Irradiated Water*

In the past fifteen years, radiation chemistry has made a large contribution to radiobiology. Most of this contribution has stemmed from one very important finding, that *irradiated water contains chemical substances that can act as cell poisons.* The hypothesis constructed around this finding was that, since living cells are in general surrounded by and contain water, the chemical poisons arising in irradiated water could be the main cause of cell death. A number of the toxic substances occurring in pure water have been identified with some degree of certainty, and the biologically important molecules are thought to be the $OH\cdot$ radical, the $H\cdot$ radical, the $HO2\cdot$ radical, and hydrogen peroxide $H2O2$. The dot following the first three formulae indicates that they are free radicals, which are very reactive chemically. In particular, the hydroxyl radical ($OH\cdot$) is a strong oxidizing agent, as are hydrogen peroxide and the peroxyl radical ($OH2\cdot$), and it is by virtue of this property that these agents are thought to be toxic for cells (1). In the presence of organic materials, as are found in cells, organic peroxides are formed as well as hydrogen peroxide, and it has been shown that under certain conditions the addition of chemically produced organic peroxides to the milieu of living cells can lead to toxic effects (5). A more thorough discussion of the role of free radicals in radiation biology is given in Dr. Hunt's paper.

It is unlikely that free radicals *produced in water* are responsible for most biological effects of radiation, because it has been found that many substances that are the normal constituents of cells will react with these free radicals and destroy them before they can act (6). At present, the "indirect effect" of radiation, due to production of poisons in water, is considered to

be less important than the direct effect of radiation on important macromolecules in the cells. This takes us into the field of molecular biology.

## III. MOLECULAR BIOLOGY

*Macromolecules With Important Functions in the Cell*

A basic concept in modern biology is the idea that cells contain a number of cell constituents of prime importance which are *macromolecules* having very large molecular weights of from several thousands to several millions. These very large molecules are usually present in each cell in relatively low numbers. Examples of such key macromolecules are the enzymes, a diverse group of proteins which catalyze the multitude of delicately balanced biochemical reactions that allow life to go on in a cell, and the nucleic acids, of which there are two kinds, deoxyribonucleic acid (DNA) which is the key substance of which genes are almost certainly made (7), and ribonucleic acid, which has an important role in the synthesis of proteins and seems to act as a messenger from the seat of management in the cell, the nucleus, to the workshop of the cell, the cytoplasm (8).

*The Target Theory*

These key macromolecules, which are so important to the cell, could be critical targets for the action of radiation. In point of fact, the existence of such targets was postulated before any really clear ideas existed about just what these targets might be. The way in which this came about was as follows: it is well known from the work of radiation physicists, as discussed above, that ionizing radiation acts by depositing energy in a material in the form of excited and ionized atoms. The electron ejected from an atom when it is ionized can in turn ionize other atoms, and in fact, most of the ionizations produced by radiation arise in this way, *along the tracks of charged particles.* Because the ionizations occur along rather well defined tracks, there are relatively large regions in an irradiated material that escape the effects of irradiation, because no charged particles have traversed them. This provided the basis for the so-called "target" theory of radiation action (1). It was postulated that a cell could be killed only if a charged particle passed through a critical target region in a cell and deposited an ionization in that region. This constituted a "hit" Ionizations elsewhere in the cell or outside the cell had no effect and were scored as misses. Thus, the chances of hitting the target area in the cell and killing the cell were considered to be just like the chances of hitting the bullseye of a target with a rifle bullet. The smaller the target, the less the chance of scoring a hit. This idea has gained a wealth of experimental support showing that in order to obtain an equivalent biological effect, more radiation must be given to a small biological entity than a large one (see for example Fig. 1 in Dr. Hunt's paper).

The development of the target theory had made a very important contribution to radiobiology. Its language and concepts are widely used, and provide a very convenient theoretical framework for the comparison and analysis of experimental results, as will be shown in Dr. Hunt's and Dr. Whitmore's papers. However, attempts to show that the target theory is correct *in detail* have not always met with success, and a number of obvious discrepancies exist. In fact, it might safely be said that the urge to prove target theory incorrect has served as a stimulus for the carrying out of quite a number of important experiments! One phenomenon that cannot readily be explained on the basis of the target theory in the *oxygen effect.* In a wide range of biological materials, ranging from enzymes to mammalian cells, the presence of gaseous oxygen increases the radiosensitivity of the material by a factor of two- to three-fold over that observed in the absence of oxygen (9). It is difficult to see how gaseous oxygen could cause a three-fold change in the size of a target!

*Free Radicals in Biological Macromolecules*

A prevalent view now held is that critical "target" macromolecules do indeed exist in the cell, particularly those comprising the genetic apparatus of the cell, but that it is possible to invluence the effect of radiation on these molecules *at the time the ionizations are produced.* It is known that free radicals can be found in biological marcromolecules ionized by radiation, even in the absence of water (10). These directly produced radicals, which can be detected by the method of electron spin resonance, as Dr. Hunt describes in his paper, can undergo chemical reactions with many substances, including gaseous oxygen. Though little is yet known about the reactions that occur, the free radicals are now thought to be the intermediates in the effect of radiation on biological macromolecules. in a very short time (much less than 1 millisecond) after these radicals are produced, the reactions they undergo can be influenced, so that the damage they impart to the molecules can be altered (the molecules are always damaged, and never, except by indirect means, made more active by radiation).

As soon as the radicals undergo reaction, the damage that they cause to the molecule is probably permanent. If this is so, it is difficult to explain how recovery from radiation damage can occur minutes or hours after irradiation. It is known that this can happen; in his paper Dr. Whitmore describes a type of recovery that takes place in irradiated mammalian cells over a period of several hours. If these cells contain permanently damaged macromolecules, then it is necessary to postulate that it is the *manifestation* of this damage, i.e., the way that the damage to the macromolecule effects the functional capacity of the cell as a whole, that can be modified after the irradiation is finished. This brings us into the realm of cellular biology.

## IV. CELLULAR BIOLOGY

*Biochemistry and Genetics*

Two important advances that have occurred in cellular biology in the last decade have left their mark on radiobiology. One is the remarkable convergence of biochemistry and genetics which has occurred, mainly through work with micro-organisms, particularly bacteria and, in fact, particularly one bacterial species, Escherichia coli (11). The other has been the development of the techniques of the techniques of tissue-culture to the point where the behaviour of single mammalian cells can be studied under near-optimal experimental contitions (12). These will be considered in turn.

Chiefly from work with micro-organisms, it has been discovered that there is an important relationship between DNA, RNA and protein in a cell. DNA appears to act as the "blueprint" in that the arrangement of the sub-unit nucleotides along the helical backbone of the fibrous DNA macromolecule constitutes a "code" which somehow specifies the functions that the cell will be capable of performing and the proteins that it is normally able to make. RNA seems to be the middleman that translates the code into a series of instructions that the rest of the cell can understand, and in fact also carries out at least some of the instructions so that the proper proteins are made by the cell. To close the circle, some of the proteins made in this way are the enzymes that in turn are needed to make new DNA and RNA. The detailed pattern by which this process, so crucial to life, goes on, is not yet understood but the broad patterns are emerging (13). The science of genetics is concerned with the changes that sometimes occur in DNA, perhaps because of a mistake occurring when new DNA is made which causes different proteins to be synthesized and the cell to function in an unusual manner. When this happens, a mutation has occurred. The interesting fact from the viewpoint of radiobiology is that it has been known for many years that *radiation* can induce mutations (14). From recent work, it is now known that the probability that a mutation will occur depends to some extent on the treatment received by the cell *after* it has been irradiated. Experiments with bacteria have shown that, for certain mutations induced with ultraviolet light, the mutation is less likely to occur if protein synthesis is inhibited immediately after irradiation. The occurrence of these mutations also appears to depend on the nature and extent of RNA synthesis that occurs following irradiation. In fact, the mutation is not fully established until DNA synthesis has occurred in the irradiated cell. These studies are incomplete, but they illustrate the way in which the manifestation of radiation damage can depend on the biochemical steps which form a key part of the life cycle of a cell (15).

*Tissue-culture*

Attempts are now being made to extend this type of analysis to mammalian cells, using, in the main, the powerful new techniques of tissue-culture, some of which Dr. Whitmore describes in his paper. It has been found that the amount of radiation required to stop continued cell division in mammalian cells, including human cells, is only about one-fiftieth of that required for the same effect on bacterial cells. The destruction of reproductive capacity in mammalian cells by radiation is thought to be due to mutations and structural changes produced in the cell nucleus (16). Chromosomes are very easily damaged by radiation, and this led to the thought that perhaps radiation interferes with the synthesis of DNA in mammalian cells. Though this question is not fully settled, there does not appear to be any important effect of radiation on the biochemical pathways of DNA synthesis in cells in tissue-culture. Most of the changes in the cell cycle due to irradiation seem to be produced indirectly by the delay in cell division that is a very general consequence of irradiating cells (17). (This work is discussed in Dr. Whitmore's paper). The biochemical mechanism underlying the effect of radiation on chromosomes is still being sought.

The fact that the reproductive capacity of mammalian cells is relatively easily destroyed by radiation has far-reaching consequences for irradiated mammalian tissues, which is the realm of histology and pathology.

## V. HISTOLOGY AND PATHOLOGY

*Cell Turnover*

There are certain tissues in the body with a high rate of cell turnover, where many cell divisions normally occur, tissues such as bone marrow and intestinal epithelium. Since cell division is easily blocked by radiation, these tissues are the first to suffer. The importance of the radiation effect on bone marrow can easily be demonstrated in a laboratory animal such as the mouse since doses of radiation that would otherwise be lethal due to marrow failure do not cause death of the mouse if it receives a transplant of unirradiated, healthy marrow cells from a very closely related mouse (18).

The effects of radiation on several different tissues are described in Dr. Bruce's paper.

*Radiobiology and Radiotherapy*

The principle that tissues with a high rate of cell turnover are the first to be affected by radiation also explains in a general way why rapidly growing cancers can be destroyed by radiation. However, it must be borne in mind that the same radiation that prevents multiplication of cancer cells also destroys the proliferative capacity of normal cells, so that care must be taken to irradiate as few normal cells as is possible. This illustrates an advantage that radiation therapy possesses over chemotherapy. The radiation beam can be directed towards the tumor, so that normal multiplying tissue is spared, while drugs, unless confined to an extremity, must circulate within the body and be available to all dividing cells. Chemotherapy will probably not replace radiotherapy until a drug is found which is specifically taken up by tumor cells. Until that time comes, radiation will continue to be a very important means of cancer therapy and radiobiologists will continue to seek ways of specifically increasing the effects of radiation on tumor cells.

The main role that radiobiology can play in the practice of radiotherapy is to search for the basic mechanisms underlying the empirical findings of radiotherapists. Accumulated clinical experience has shown these findings to be valid. The effects of dose-fractionation, and the existence of tumors which respond well to radiotherapy and tumors which respond badly, are examples (19). The radiobiological basis for these findings is being sought with increasing success. Some of these problems are considered in more detail in Dr. Bruce's paper.

*Radiation and Society*

The most important effect of radiation on the population as a whole is probably the genetic effect, that is, the induction of mutations, the vast majority of which can be expected to be harmful (20). Contrary to what appears to be the popular view, radiation is not a purely modern phenomenon. There is nothing new about the hereditary effects of radiation; they have always been with us. The rocks of the earth contain naturally radioactive elements, such as radium, so that the walls of a stone house contain radioactivity. Our own bodies contain the naturally radioactive isotope of potassium, $K^{40}$, in amounts large enough to be detected by sensitive instruments. The atmosphere is being continually bombarded by ionizing radiations from cosmic rays which are far more powerful than any man is yet able to produce. All these radiations add up to what is called "background" radiation, and this is responsible at the present time for about half the ionizing radiation to which the reproductive tissues of the general population are being exposed. This has been and is being increased by other, man-made, sources of radiation, including the medical use of x-rays (which is by far the next biggest contributor of radiation, roughly equal on the average to that due to background radiation) and fallout from nuclear bomb tests (which contributes on the average about 2½ percent of the total radiation dose received by the reproductive tissues). In addition to these, there are several other sources of radiation of, at present, minor importance, such as radioactive wastes from the nuclear reactors used in nuclear power plants, atomic piles, nuclear ships and submarines etc.; radioactive isotopes used in science and medicine; radiation from radium-dial watches and television sets, and exposure to increased background due to high altitude jet-aircraft travel. Some of these are certain to increase in importance, but vigorous attempts should be made, and are being made, to keep unnecessary exposure to man-made radiation at a minimum.

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# THE INITIAL ACTIONS OF IONIZING RADIATIONS

J.W. HUNT, Ph.D.

From the moment that radiation falls on a living cell until the time that some permanent damage is produced, a number of rapid reactions take place. These *begin* with the absorption of energy from the radiation beam, and *end* with the damage to some critical cellular function. From beginning to end, these initial reactions are probably finished in a millionth of a second; this makes them very difficult to study, especially in the relatively heterogeneous interior of an intact cell. However, by using modern techniques and simpler biological systems, it is possible to learn much about the initial actions which take place immediately after the absorption of the radiation. The processes and reactions which occur in this initial microsecond interval will be described in this paper, with special emphasis placed on studies using some of the newest physical techniques.

Ionizing radiations are of great interest to us, partly because they include the x-rays and gamma-rays used for medical diagnosis and therapy. For the sake of simplicity, the effects of other radiations such as ultra-violet, light, and radio waves will not be discussed here. In addition to x-rays and gamma-rays, beta-particles (electrons), alpha-particles and high speed protons or neutrons are all ionizing radiations. When these radiations pass through any material, they lose energy and in this process produce ions along their path. The ions that are produced by radiations are very different from the stable positive and negative ions formed by the dissociation of salts in solution. The radiations produce ions by knocking electrons out of molecules that lie in the radiation beam; these molecules, lacking an electron, are charged ions. The number of ions which are produced in a material is directly proportional to the radiation dose it receives. The radiation beams of x-rays, gamma-rays and beta-particles are found to be criss-crossed haphazardly by electron tracks, each track leaving a string of ionized particles in its wake. Heavier particles, such as alpha-particles and high energy protons or neutrons, produce tracks which are similar to electron tracks except that the density of the ions along them is much greater. This results in some alterations in the details of their radiation actions, but the overall picture remains unchanged. The immediate action of radiation, therefore, is to form strings of ionized particles throughout the medium.

## MECHANISMS OF RADIATION DAMAGE

Two general theories have been proposed in an attempt to explain the initial actions of radiation in producing damage. The first is the theory of direct action, which proposes that damage is caused by those ions which are formed in some critical target in the irradiated material. The second is the theory of indirect action, which tries to explain the effect of the irradiated medium surrounding the target in enhancing the radiation damage. The mechanisms of damage proposed by both these theories are not contradictory; in fact, both mechanisms are important in the production of radiobiological damage.

### *Direct Action*

The theory of direct action, upon which the target theory was developed (1,2), implies that some critical molecule or region undergoes a change as the direct result of one or more ionizations within it. In the simple case of a dry enzyme molecule, it has been found that a single ionization *anywhere* in the molecule constitutes a hit in the target, and results in the loss of its biochemical activity. Moreover, one would expect that the larger the target molecule, the easier it would be to hit. Using basic mathematical concepts, it is possible to calculate the target size from the number of unaltered molecules which remain after a certain dose of radiation. For gamma-irradiation of small molecules, the target size or sensitive volume is given by V-0.7/Do where V is the volume of the target in cubic microns and Do is the dose in rads needed to produce the average of one ionization in each target (2). If the hits are randomly distributed, this is the dose which leaves 37 percent of the molecules undamaged. With many refinements, this technique has been used with singular success by Lea (2) and by Pollard's group (3), to determine the size of enzyme molecules, phage and virus particles. If one takes the radiation sensitivity, as measured by Do, for a wide range of biological materials and plots it against the size of the particle or cell, a graph such as that shown in Figure 1 results. This graph shows that, as the physical size of the biological ma-

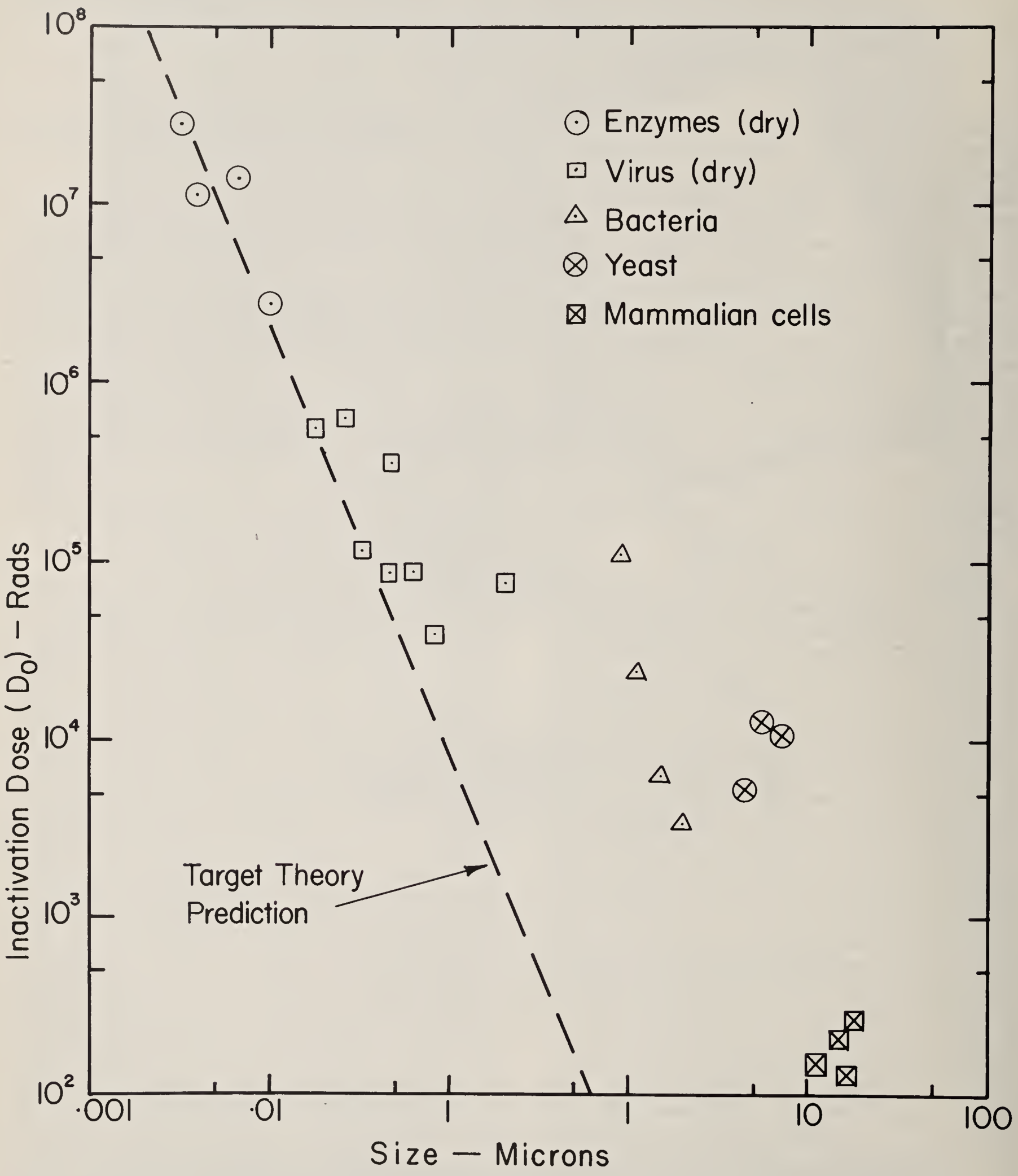


Fig. 1. A comparison between the inactivation dose Do (see text) and the size of the irradiated particle or cell. As the size of the system increases, the dose needed to inactivate it decreases, which indicates an increase in target size. The dashed line shows the target size predicted by Lea (2) for these values of Do.

terial being irradiated increases, the dose required to inactivate it decreases. The thesis that the larger the target, the easier it is to produce damage, undoubtedly applies here. For simple enzymes, the target seems to be the whole molecule, but as the size and complexity of the system increases, the target volume becomes less than the volume of the entire organism. For example, in a mammalian cell, the diameter of the critical region or "target" is about one tenth the diameter of the cell. If the theory of direct action could be applied here to a single target, it would mean that one ionization anywhere in a region a micron in diameter would kill the cell. The target theory has had its greatest success in interpreting the results of irradiations carried out on dried enzymes and viruses. However, in the presence of water, oxygen, protective agents, etc., significant deviations from these predictions occur. Some of these deviations can be explained as effects of indirect action.

*Indirect Action*

The material surroundint the target such as water for example, is also altered by the radiation. Reactive products, often called radiation poisons, can diffuse from the surroundings to attack the target and render it inactive. An enzyme in dilute aqueous solution is an excellent test of these "poisons". In solution, an enzyme is more sensitive to radiation than it is dry. If the concentration of enzyme in a given volume of solution is progressively decreased, it is found that over a wide range of concentrations, the *number* of enzyme molecules which are inactivated by a given dose of x-rays is a constant. In other words, it is the number of ionizations in the water and not hits to the enzyme molecules which determines the amount of enzyme inactivated. This shows that some reactive poison is produced in the water, which reacts, molecule for molecule with the enzyme, to destroy its activity. These poisons are thought to be free radicals; they will be discussed in greater detail later. The theory of the indirect effect has been successful in explaining radiation damage to enzymes in dilute solutions, and in combination with the direct effect this theory has explained radiation effects on viruses in solution (2). For the complex organisms, such as mamalian cells, however, it becomes very difficult to determine whether the direct or indirect effect is acting. Recent experiments indicate that energy may be transferred over a considerable distance along macromolecular chains, producing damage at a point quite distant from the original ionization (4,5). If this is true, the direct damage to a macromolecule and the subsequent transfer of energy to a sensitive region might produce almost the same end result as the attack by a radiation poison on this region.

The mechanisms of direct and indirect actions can be applied to irradiation damage under specified conditions in simple systems. The gross agreement between the radiation sensitivity and size of the system indicates that even

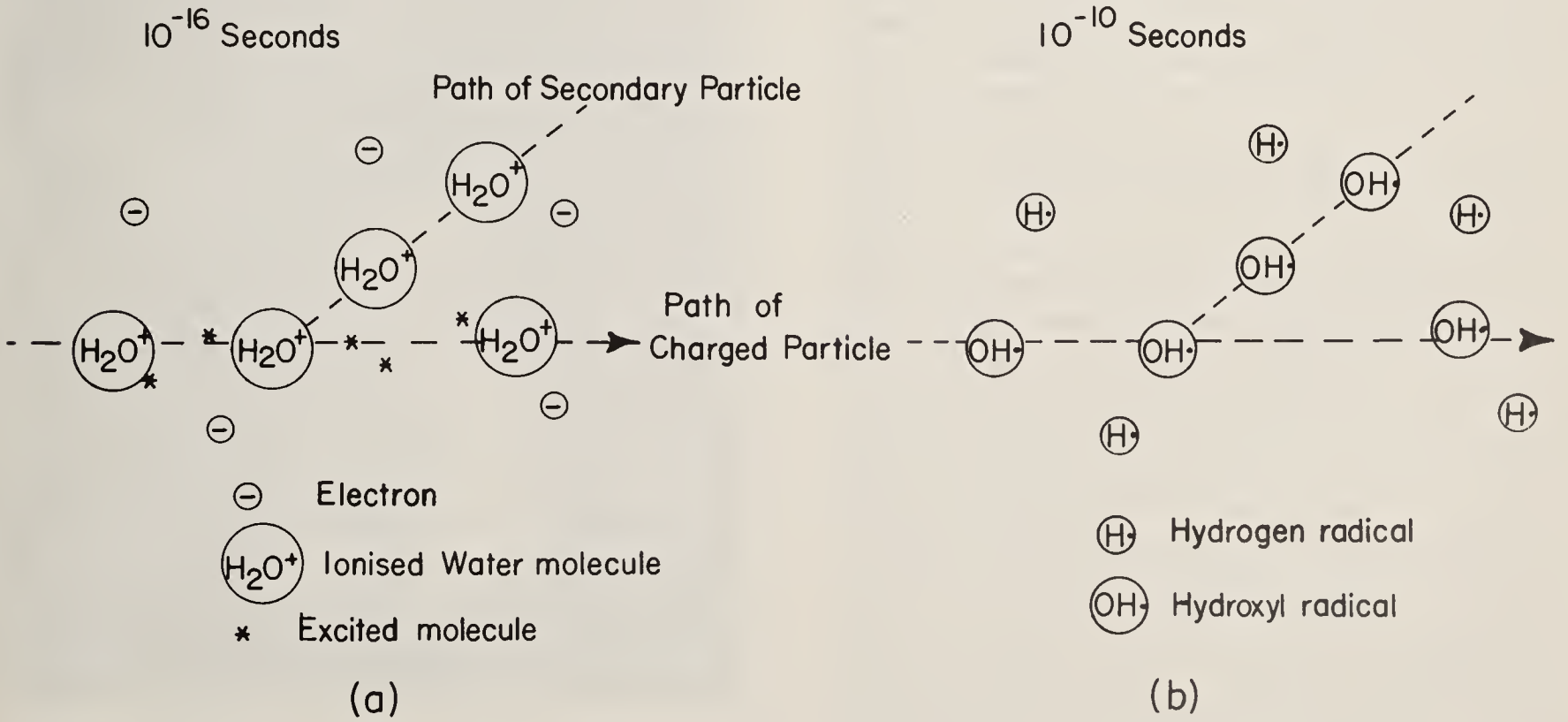


Fig. 2. The rapid reactions along the path of an ionizing particle passing through water: (a) about 10-16 seconds later, positive ions have been produced along the track by knocking out electrons, (b) rapid dissociations then take place 10-10 seconds later, producing reactive free radicals.

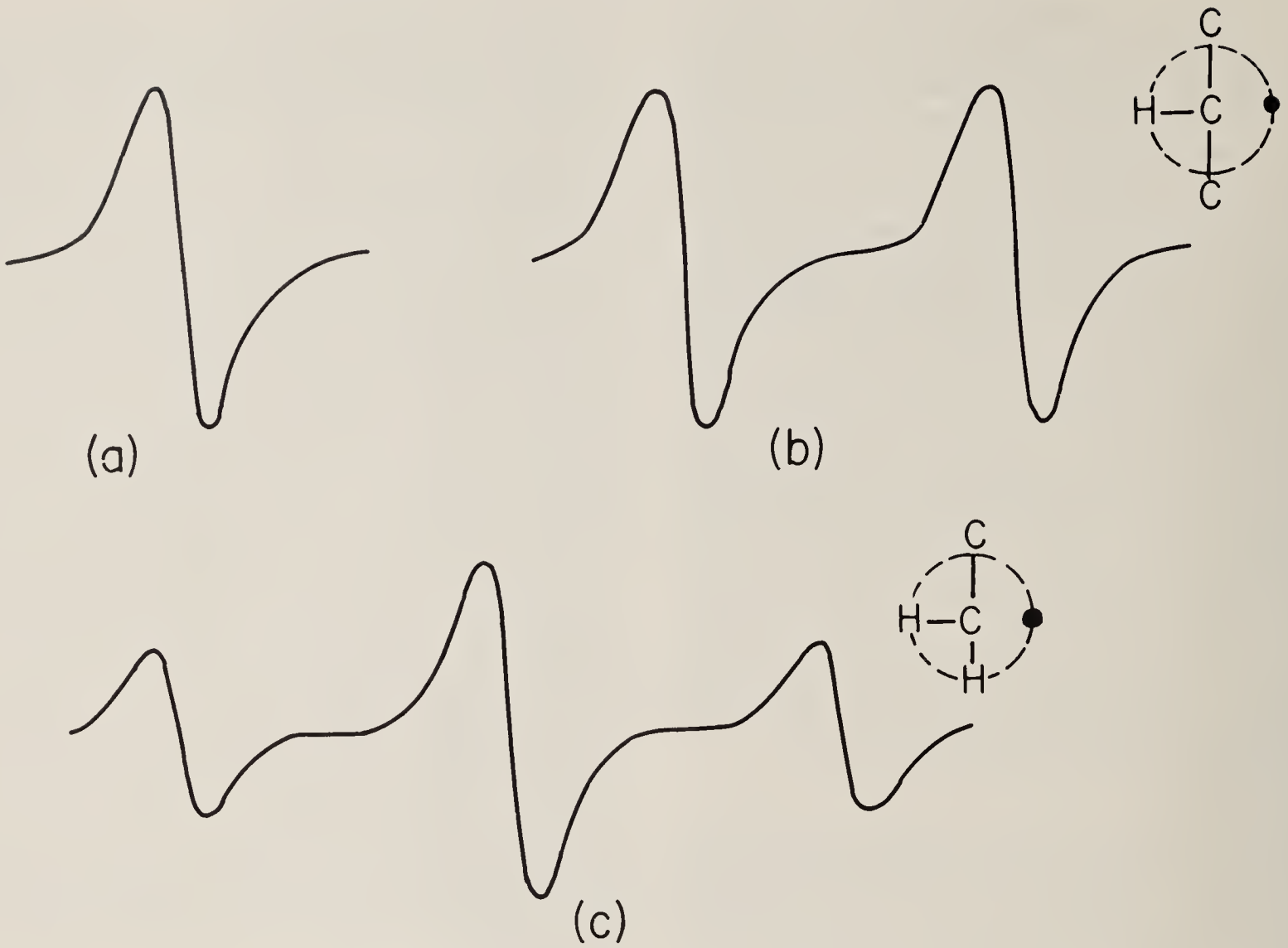


Fig. 3. The ESR spectra obtained for different types of free radicals: (a) a simple absorption line, (b) the spectrum which is obtained if the free electron is located part of the time on one hydrogen atom, (c) the spectrum if the electron is shared by two hydrogen atoms.

the free radicals in an ESR spectrometer without influencing in any way the reactions in which they are taking part. By adding a number of electronic refinements, it is possible to make these spectrometers extremely sensitive. Such an instrument is capable of detecting about $10^{13}$ organic free radicals; these radicals would weigh only about $10^{-9}$ grams! A quantity of up to a gram of material containing no unpaired electrons may be present along with the free radicals without affecting the spectrometer's ability to detect them. We have at our disposal, therefore, a new tool which is able to detect, study and sometimes identify the free radicals formed as intermediates in the process of radiation damage.

Some very interesting facts about free radicals were discovered by one of the first workers to use this new tool, E. E. Schneider, who discovered that in irradiated solids, radicals do not react and disappear with the same rapidity that they do in solution (9). Some radicals in irradiated organic compounds have almost infinite lifetimes (10) and so may be studied long after the irradiation has been completed. It is postulated that

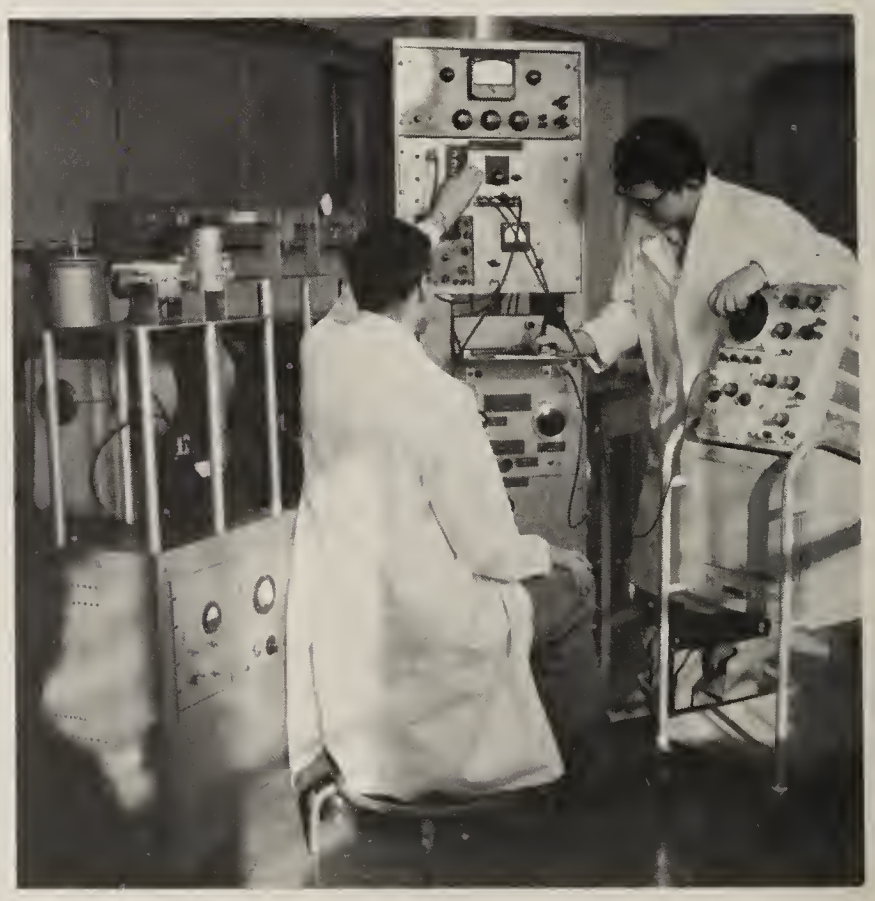

Fig. 4. An electron spin resonance spectrometer designed and constructed at the Ontario Cancer Institute for research on free radicals in irradiated materials.

for mammalian cells, the target theory may supply valuable information. At the very least it indicates the approximate size of the region through which the transfer of energy or diffusion of a poison must take place if a random ionization is to damage a sensitive key molecule.

## FREE RADICALS

This description of the initial actions of radiations has not yet covered the intermediate steps which take place between the time when the ionization occurs and the key target is damaged. The radiation chemists' views on what takes place in water are shown in Figure 2 (6). The water molecules are ionized by the passage of the charged particle, with the secondary electrons drifting some distance away, where they are eventually captured by other water molecules. The ionized water molecules are unstable and may dissociate, forming highly reactive fragments .OH and H.. Each of these fragments has one unpaired or "free" valence electron, which results in its chemical reactivity and explains the name, free radical, which has been given to it. This free electron is represented by the dot in the chemical symbol of a free radical. The reactions in water may be written

$$H_2O + \text{radiation energy} \longrightarrow H_2O^+ + e^-$$
$$H_2O^+ + H_2O \longrightarrow H_3O^+ + \cdot OH$$
$$e^- + H_2O \longrightarrow H\cdot + OH^-$$

and in the presence of oxygen

$$H\cdot + O_2 \longrightarrow HO_2$$

Recombinations of free radicals may occur, the most important reaction being

$$\cdot OH + \cdot OH \longrightarrow H_2O_2$$

These free radicals rapidly react with neighbouring solutes and with other radicals and disappear in about a millionth of a second. One important product of the radical reactions is hydrogen peroxide which has been shown to be important in some radiobiological systems (7). Both ·OH and $HO_2$. are strong oxidizing agents and thus are thought to be the radiation "poisons" of the indirect effect. These concepts have been highly successful in explaining the reactions in solutions containing inorganic ions, (8) and to a lesser extent, the inactivation of enzymes in solution.

The success of the radiation chemists in explaining some of the simpler reactions in solution led them to hope that these reactions could explain the great radiation sensitivity of the cell, which itself is mostly water. The cell, however, is far from being the purified water sample used in the radiation chemistry experiments. In the cell the radicals are likely to react with organic molecules before they have time to diffuse a significant distance and reach the critical target molecules. It is unlikely that the simple diffusion of radicals produced in water can entirely explain the extreme sensitivity of the cell to radiation damage, but these radicals undoubtedly must play an important part in the process of transferring damage to the sensitive target.

### *Detection of Free Radicals Using Electron Spin Resonance Techniques*

The part that free radicals play in the process of radiation damage has been very difficult to study because of the extreme reactivity of the radicals. Until recently, indirect chemical methods have been used to study these radicals, but now a new instrument is available which is known as an "electron spin resonance (ESR) spectrometer".

This complicated, physical technique makes it possible to detect and sometimes to identify free radicals by observing the presence of their unpaired electrons. The detection is possible because of a peculiar electromagnetic property of these unpaired electrons which is called their "spin". If a sample is placed in a suitable magnetic field, it can absorb power from a microwave (radar) beam falling on it. The absorption of microwave energy is detected by sensitive electronic means. This absorption is critically dependent upon the strength of the magnetic field, so that if the field is slowly increased, an absorption line such as the one shown in Figure 3 (a) will be seen. This characteristic absorption occurs only for free radicals and a few other unusual molecules, so normally no absorption can be observed in an unirradiated sample.

If this simple absorption pattern were all that appeared, the study of ESR would be only of slight interest, for all free radicals would then show the same pattern. Fortunately, this absorption line is usually split up into a number of components because of interactions by the free electron with neighbouring atoms in the molecule. The effects of one and two neighbouring hydrogen atoms on the ESR spectrum are seen in Figure 3(b) and (c). The spectrum is broken up into two or more components, whose separation and relative intensities are characteristic of that particular radical. The size of these absorption lines is a measure of the number of free radicals which are present making it possible to study the growth and decay of the radical species. It should be emphasized that, unlike most chemical methods of study, it is possible to observe

their molecular chains hold the radicals in place and prevent their reaction with nearby molecules.

*Detection of Free Radicals in Water*

Another method of stabilizing these free radicals was investigated by Dr. R. Livingston and his associates at Oak Ridge (11). They froze acidic solutions of water down to liquid nitrogen temperatures (-196° C) to stabilize the radicals and held the samples at the temperature while they were being irradiated. Without being warmed, they were studied on a low temperature ESR spectrometer. In fairly strong acid solutions, hydrogen atoms ($H\cdot$) were observed, but the hydroxyl radicals ($OH\cdot$), thought to be so important in biological damage, could not be found, even if the molecular motion were almost stopped by cooling the sample to liquid helium temperatures. It is still a mystery why these radicals seem to react and disappear before they can be detected.

At room temperature, these radicals are thought to have lifetimes in the order of one microsecond, and so they generally disappear before one can begin to look for them. We hope to solve this problem in our laboratory by observing the ESR spectrum of a water sample at the same time as it is being irradiated. To build up a high enough concentration of radicals during their short lifetimes, an extremely intense source of radiation is needed; fortunately this is available from a Van de Graaff generator in our laboratory, If this experiment is successful, some of the questions regarding the process of radiation action in aqueous solutions should be clarified.

## RADIATION DAMAGE TO MACROMOLECULES

In the earlier discussion, it was pointed out that the mechanism of radiation damage may be indirect, through the formation of destructive "poisons" in the cell, direct, through the ionization of some critical molecules, or a combination of the two.

The large size and uniqueness of the sensitive radiation target has led workers to suspect that some large macromolecule such as DNA may be the key to the high radiosensitivity of the cell. Considerable attention is now being focussed on the process of damage to large organic molecules in the hope that they will help to explain the process of radiobiological damage.

The large "macromolecules" in which we are interested range from "small" enzyme molecules with a molecular weight of 10,000 to the immense molecules of DNA which have molecular weights of 6,000,000 or more. Molecules of this size generally consist of polymers or other long chain structures having some repeated structure. The question is asked: how does radiation affect these macromolecules?

In the polymers of plastics, for example, two possibilities of radiation damage seem to exist. If one irradiates polyethylene, cross linked molecules are produced, presumably through the loss of a hydrogen atom from the main chain (Fig. 5) (12). This results in a cross linking between the two polymerized chains. In isobutylene, the configuration of the molecule seems to favour the breaking of the main chain into fragments (Fig. 6) (12).

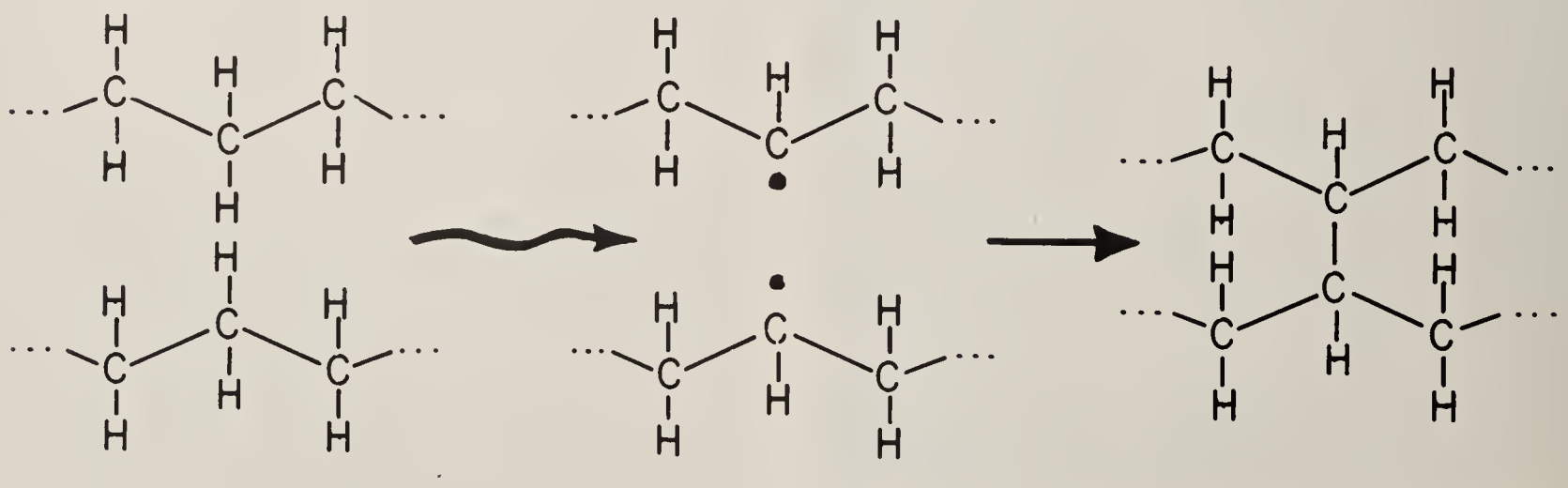

Fig. 5. The radiation induced cross linking of polyethylene. Free radicals are formed on two adjacent chains. These combine to form a cross link between the macromolecular chains.

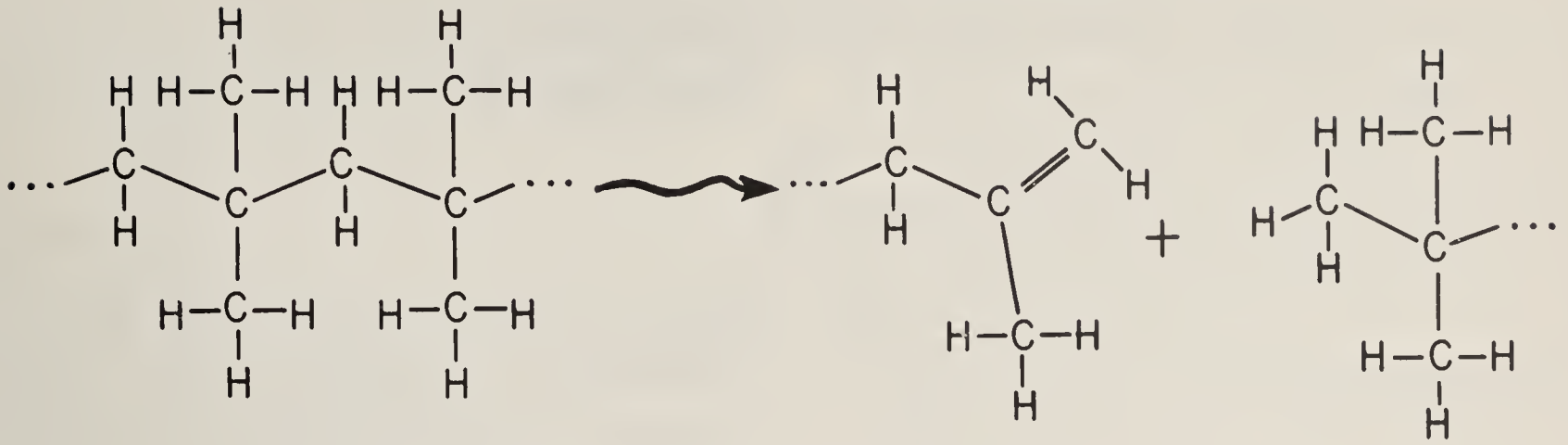

Fig. 6. The breaking of isobutylene chains by radiation.

*Proteins*

In more complicated macromolecules, such as one finds in proteins, there are many possibilities for the production of a variety of radiation effects. The ESR spectra of dry irradiated proteins, however, all show a doublet line structure of remarkable similarity (13). These spectra probably mean that the free electron is associated with a hydrogen atom, as shown in Figure 3 (b). By carefully studying the spectrum from irradiated silk, Gordy (14) has been able to conclude that the principal radiation lesion is the removal of a group attached to the main polypeptide chain (Figure 7).

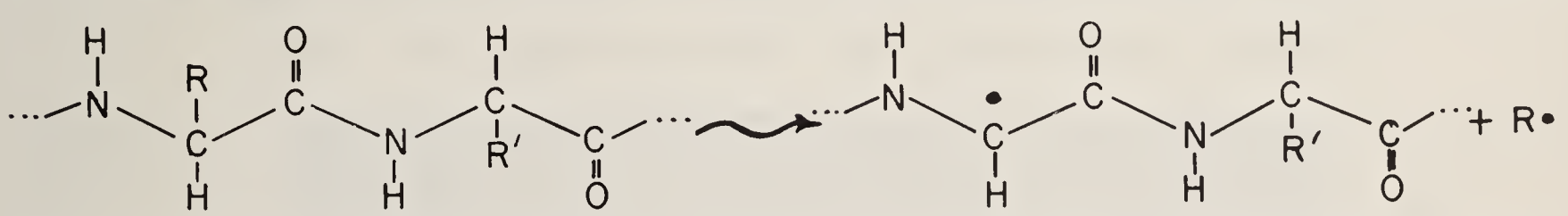

Fig. 7. The process of radiation damage to a protein as postulated by Gordy et al (13) from ESR studies. They postulate that the radiation selectively removes a group, R, from the polypeptide chain and produces a free radical.

Other studies using serum albumin (15) indicate that main chain breakage occurs as well as alteration in the amino acids in the polypeptide chain.

*Enzymes*

Enzymes, a special class of proteins, are especially suitable for radiation study since a suitable yardstick of damage may be obtained from their biological activity. The radiation sensitivity of enzymes has been studied for many years, but our interest in the field was recently kindled by the fact that it is now possible to study the free radicals produced by the radiation. We wanted to see if correlations between the radiation damage and the ESR spectrum could be obtained.

For this study we have chosen the enzyme, ribonuclease (RNase), which we consider particularly suitable for the investigation. This comparatively small molecule with a molecular weight of 12,066 has been the object of intensive study be a large number of workers, so that its amino acid sequence and general structure is known. Its biological activity may be conveniently assayed by a number of techniques (16). The enzyme is irradiated in the form of a dry, polycrystalline solid so that no indirect action takes place, and the free radicals are stabilized.

The ESR spectrum of ribonuclease irradiated in vacuum, or in nitrogen, shows the two unresolved peaks characteristic of most proteins (Figure 8 (a)). If oxygen is present during the irradiation, the spectrum is radically changed to form a single, narrow peak (Figure 8 (c)). This change is accompanied by an increase of 2.3 times in the radiation sensitivity of the enzyme, which is approximately the same enhancement as observed in a wide range of biological systems irradiated in the presence of oxygen. (17) A rather surprising finding is that oxygen need not be present during the irradiation in order to enhance the sensitivity. The same result is obtained, both in the changed ESR spectra and in the enhanced sensitivity, if oxygen is added at any time before the enzyme is dissolved in order to measure its activity. As soon as the enzyme is dissolved, the free radicals rapidly react and the damage is fixed in its final form. If nitric oxide is present during the irradiation, the free radical spectrum completely disappears (Figure 8(d)). This gas protects the RNase slightly from the radiation damage. The presence of a small

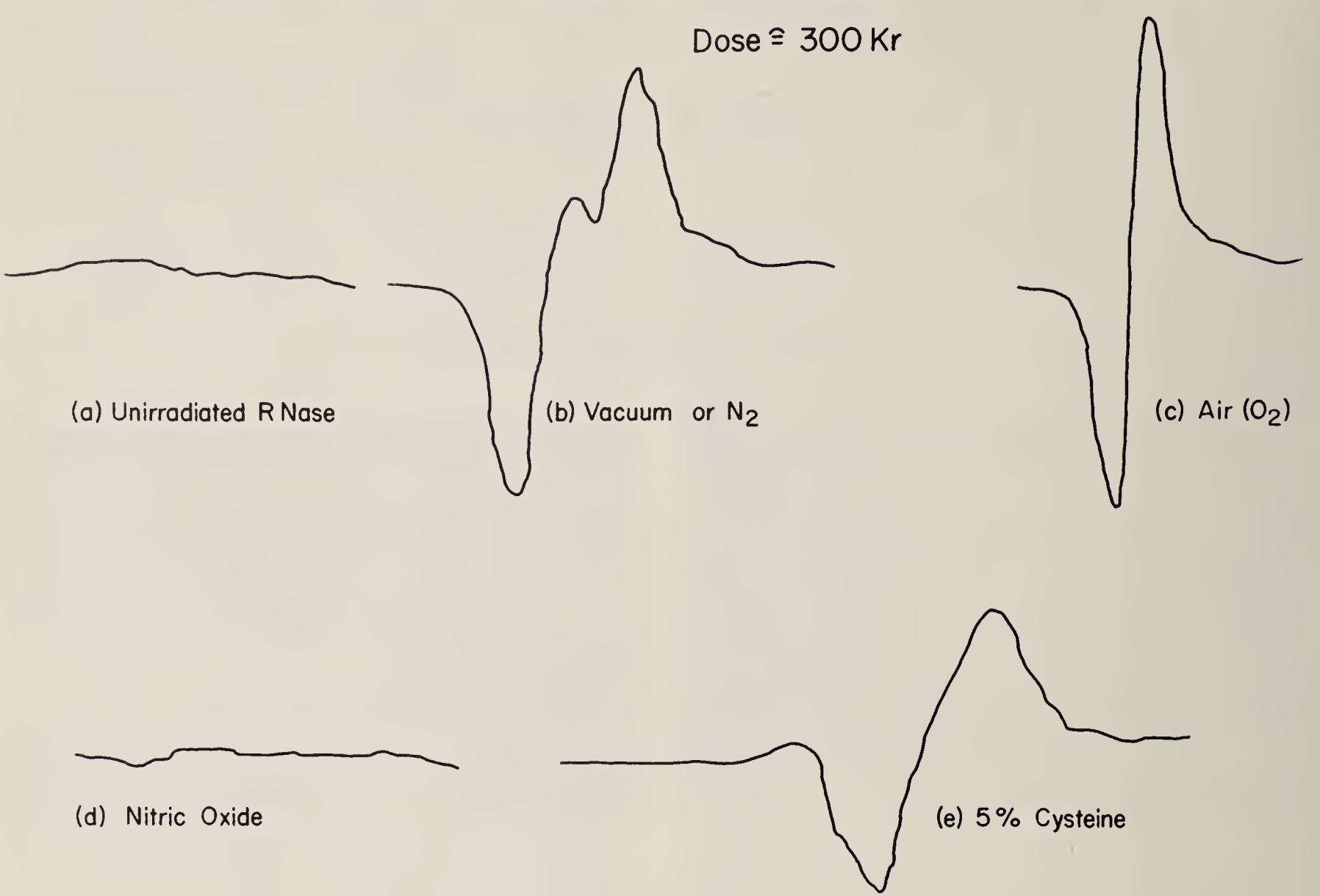
E. S. R. Spectra for Irradiated Ribonuclease.
Dose ≅ 300 Kr
(a) Unirradiated R Nase
(b) Vacuum or $N_2$
(c) Air ($O_2$)
(d) Nitric Oxide
(e) 5% Cysteine

Fig. 8. The ESR spectra of the free radicals in irradiated ribonuclease: (a) unirradiated ribonuclease, (b) irradiated in vacuum or in N2, (c) O2 present, before or after the irradiation, (d) irradiated in NO, (e) 5 percent cysteine present in the ribonuclease sample.

quantity of the well known protective agent, cysteine, also considerably alters the spectrum (Figure 8(e)). It appears that the radiation damage, as indicated by the free radicals, can be transferred in part from the RNase to the cysteine molecule.

These results agree with the thesis that radiation damage exists for a time in the form of free radicals, and that reactions by these radicals result in the final fixing of the damage. It appears that oxygen and protective agents seem to act on these transient free radicals.

It is very interesting to note that the radiation damage to ribonuclease and to dry bacterial spores (18) both vary in the same way with different gaseous conditions. This indicates that either protein damage may be an important link in the chain of biological damage, or we may be dealing here with some universal reactions in irradiated biological systems. If either hypothesis is true, the detailed study of such simplified systems will undoubtedly give valuable information concerning the process of radiation damage in living cells.

## SUMMARY

The initial process of radiation damage has been described beginning with the formation of ionized molecules along the tracks of the radiation particles. These ions immediately dissociate to form free radicals, and the free radicals react to produce permanent damage to some key molecules. The tracks of ionized molecules may pass through some sensitive region or "target" in which a single ionization may inactivate the molecule, the particle or the cell. The larger the target, the easier it is to hit; the dose required for inactivation decreases with increasing target size. The role which free radicals play in radiation damage is being investigated using the new technique of electron spin resonance. Experiments indicate that changes induced in these free radicals will alter the course and extent of

the radiation damage. The problem of the high sensitivity of cells to radiation damage may be solved by a further understanding of the initial actions of radiation.

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# RADIOBIOLOGY AT THE CELLULAR LEVEL

G.F. WHITMORE, Ph. D.

Within six months of the discovery of x-rays by Roentgen in 1895 it was found that these rays had the ability to kill cells. This fact constitutes the basis for the present day treatment of malignant tumors by radiotherapy. However, while radiotherapy has made great strides in the years since 1895, and whereas our understanding of many of the physical processes whereby energy is transferred from the radiation beam to a biological system is now largely complete, the central problem of radiobiology still remains and may be stated. How is it that the small amount of energy imparted to any biological system during the course of a lethal irradiation is able to bring about the death of that organism whether it be a whole body or a single cell, or even a bacterium or a virus? Some idea of the minute amount of energy involved can be obtained from the realization that the energy absorbed by a single mammalian cell during the course of a fatal irradiation is less than the energy which would be required to carry out the normal life processes of that cell for a period of one second.

While this is the fundamental question of radiobiology, it is not likely to be answered in a definitive way for perhaps many years. There are, however, other very pertinent questions which are of concern to the radiotherapist and radiobiologist which can perhaps be answered with present day techniques and which will give us at least partial answers to the primary problem stated above. Some of these questions, which apply to mammalian cells, can be stated as follows. Are all types of mammalian cells equally sensitive to radiation or more specifically, are all types of cells within a single mammalian species equally sensitive to radiation? Is it possible to alter the radiosensitivity of various cell lines to make them either more sensitive or more resistant to radiation damage? Can cells recover from radiation damage or is such damage necessarily permanent? What metabolic changes occur in an irradiated cell which may bring about its death from radiation damage or which may enhance its chances for recovery?

All of these problems are under active study in various laboratories throughout the world and it is the purpose here to describe some of the methods which are being applied, some of the answers which have been obtained, and some of the more detailed questions which have arisen as a result of this new knowledge. Space limitations prevent a complete survey of all radiobiological research at the cellular level and therefore the following discussion will be limited to some results which have been obtained from studies on mammalian cells grown and irradiated *in vitro,* that is, results obtained by the use of tissue culture techniques. Because of the danger of blind extrapolation from tissue culture results to results *in vivo,* mention will also be made of several *in vivo* experiments which seem to yield results which can be compared with *in vitro* studies.

## TECHNIQUES TO DETERMINE RADIATION SENSITIVITY

It is now relatively easy to remove many kinds of living cells from a mammalian organism, transfer these cells to an *in vitro* situation, and with the use of complex, but largely chemically defined growth media maintain the growth of these cells over periods of weeks or months. There are now certain "established culture lines" which will grow indefinitely in tissue culture. Most fresh explants of cells will grow only when the cells can adhere firmly to some surface which is usually glass or plastic, and overlaid with growth medium. Certain established cell lines, notably the L cell, will however grow in suspension in much the same way as bacteria will grow in suspension. Under conditions of suspension growth, it is possible to obtain cell cultures containing close to one million cells per cc and in these suspensions each cell is floating free of its neighbours. (Fig. 1.).

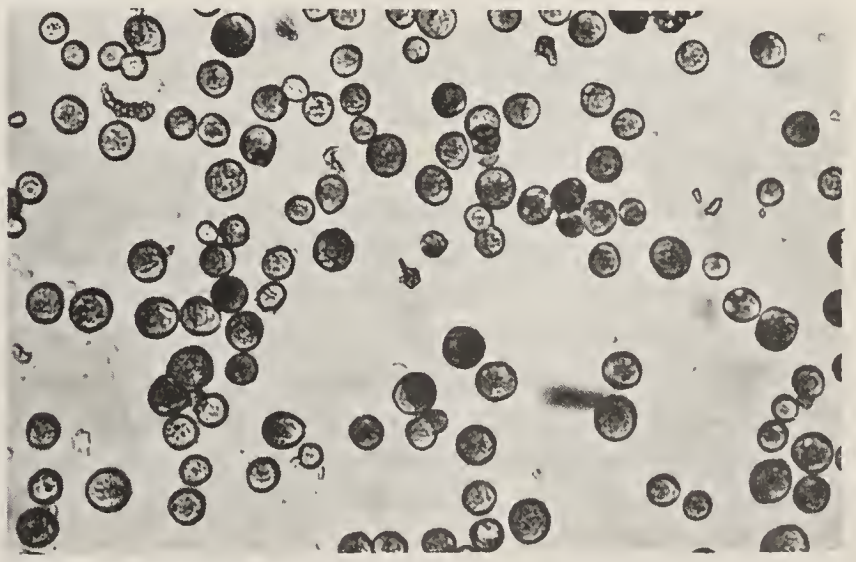

Fig. 1. Photomicrograph showing L cells grown in suspension culture.

Such a population of cells will double in number every twenty-four hours if the growth medium is kept replenished and the number of cells per cc does not exceed a certain critical value. If cells from suspension culture are transferred to stationary cultures, either in petri dishes or in bottles, then each living cell will settle out of the medium, attach to the glass and begin to multiply and as a result each cell ultimately gives rise to a visible clone, or colony, of cells with the cells in each clone or colony being derived from the single parent cell that settled out in that position (Fig. 2).

Fig. 2. A group of culture bottles exhibiting various numbers of clones or colonies. The x-ray dose given to each bottle increases from left to right (see text).

This procedure provides a very convenient way for determining the number of cells in any cell culture that are capable of proliferation and hence this procedure is admirably adapted to studies which are concerned with ascertaining the effects of radiation in arresting cell division in cells. Cells that are shown to be capable of forming clones or colonies by the method described above are by common usage said to be *viable,* and by determining the relative numbers of cells in a culture that are viable and non-viable what is said to be the *radiation sensitivity** of the cells under study can be determined. A brief outline of a typical experiment of this kind will now be described.

Each of several samples of cells in a suitable fluid medium, each sample containing the same number of cells of the same type, is exposed to a different dose of radiation. After the radiation the cells of each sample are transferred to cultures which permit the cells to settle out on some suitable surface. The cultures are then incubated for 14 days and during this time each cell that is still capable of proliferating has time to multiply often enough to give rise to a clone containing between 1000 and 10,000 cells. Such a clone, when it is stained, is visible to the naked eye. The appearances presented by cultures of five different samples are shown in Fig. 2. The one at the left was given no radiation, and each of the others, from the left to the right, received increasing amounts of radiation. It is obvious that most of the cells in the right hand culture have been killed. However, about 5 percent of the cells have survived the radiation. If one plots the number of cells which remain alive versus the radiation dose, then one obtains a "survival" curve for the particular type of cells and the particular type of radiation used in the experiment. This survival curve can then be used to predict the number of cells in a population which whil survive any given dose of radiation. The survival curves for a number of cell types will also enable us to determine whether all types of cells have the same radiosensitivity and if not, over what range variation exists.

* It should be pointed out that this is a restricted definition of cell sensitivity since it is only concerned with the cells' ability to divide. Another type of experiment might be designed to measure the radiation sensitivity of a cell's ability to synthesize a hormone or similar substance. In this case the radiation sensitivity might be very different from the radiation sensitivity of the cell's ability to divide. For this reason when talking about radiation sensitivity the cell process being measured must be stated or the discussion is meaningless. In the sections to follow radiation sensitivity will only be concerned with the cells' ability to proliferate.

## TARGET THEORY AND ITS APPLICATION TO SURVIVAL CURVES

Because the shape of the survival curve can often be used to tell something about the nature of the x-ray sensitive "target" a brief digression to talk about target theory and its possible relation to survival curves might be in order. The concepts of target theory have been discussed by Dr. Hunt but certain ideas will be expanded here.

Much of the language of radiobiology has developed from the concept of target theory, which, in its simplest forms, visualizes a special "target" or targets within a biological system such as a cell, which if "hit" by radiation, will result in serious biological consequences to the whole system. It is therefore customary to speak of either single or multiple targets and of "lethal" hits. A lethal hit would occur when an important target in a cell was hit. It must be borne in mind that the use of the words target and hit must not be taken to mean that the nature of the target is always known or that the exact nature of the reactions that follow a hit are known.

Perhaps the easiest way to visualize target theory is by means of an analogy. Since radiation comes in the form of discrete bundles of energy or "photons" which pass in randomly distributed tracks throughout the irradiated volume, it is somewhat similar to a blind man randomly throwing balls at milk bottles in order to win a prize. In order to win a prize (that is, hit a target) he must knock over any one milk bottle. His chances of winning a prize will be determined by (1) the number of balls he is allowed to throw, (2) the size of the balls and (3) the sizes of the milk bottles and the area over which they are distributed. By analogy the dose of radiation corresponds to the number of balls thrown; the type of radiation, whether it be x-rays, electrons, high energy protons or neutrons, corresponds to the size of the balls that are thrown and the size of the biological target whether this be a single gene, a chromosome or an entire nucleus corresponds to the size of the milk bottle. For the biological system it is possible to construct a type of survival curve that would, according to our analogy, correspond to the number of milk bottles which remain standing versus the number of balls which have been thrown.

This analogy illustrates the simplest type of a target theory calculation and, from the blind man's point of view, the simplest form of this game of chance. However, in the irradiation of biological materials and in most games of chance, things are not likely to be this simple. The operator of the game, instead of spreading the milk bottles at regular intervals, would be more likely to put his milk bottles into groups in such a way that a ball would be unlikely to knock over all the bottles in one group. Furthermore he would now require that all of the bottles in any one group be knocked over before he would part with a prize. This would make the game vastly more difficult and in fact the chance of winning a prize would go down roughly as the power of the number of the bottles in each group. If there were two bottles per group, then the blind man's chances of winning a prize would be about one quarter of what they were with one bottle per group, In this game, a "survival curve" is constructed, not by counting up the fraction of the bottles which remain standing, but by counting the fraction of the groups in which one or more bottles remain standing and plotting this versus the number of balls thrown.

The above roughly represents the situation which exists when radiation acts on certain biological organisms. These organisms may contain two or more identical targets per organism, and these targets are separated in such a way that it is difficult for all of them to be inactivated by a single radiation event. As long as any *one* of these identical targets remains undamaged, the organism will survive the irradiation. Under these conditions, the shape of the survival curve will depend on the number of identical targets per organism and the size of each individual target. As examples of this type of target one could visualize two identical chromosomes in a single cell; as long as either one is functional, the cell will survive.

Rather than attempting a mathematical derivation of the various shapes of survival curves, it may be better merely to refer to Fig. 3 which illustrates the two most common forms. The first

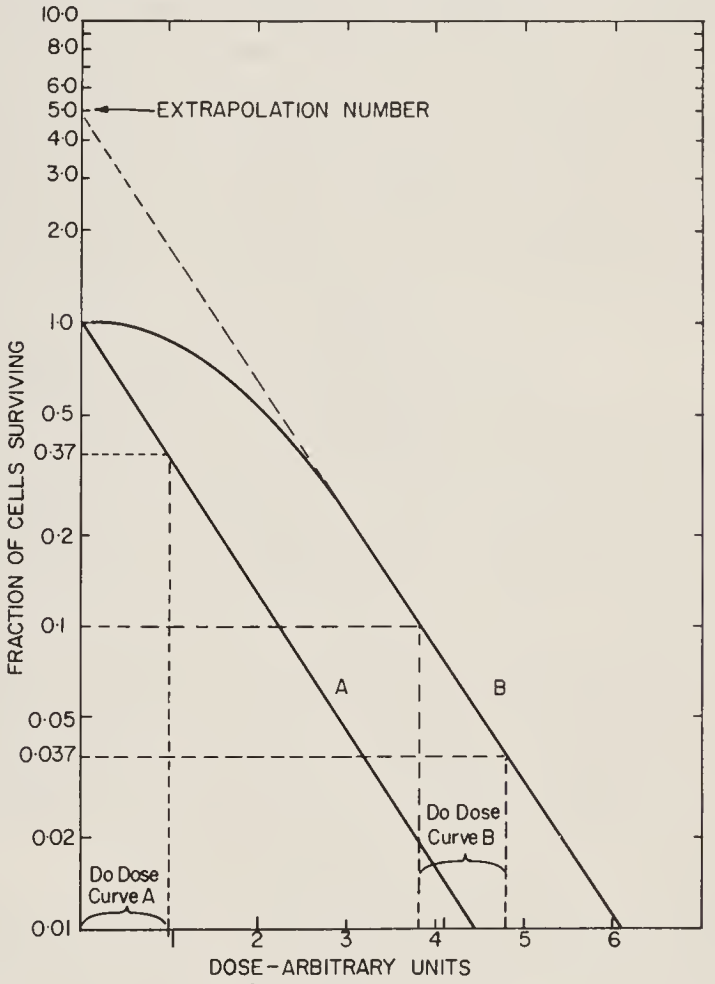


Fig. 3. Two types of radiation survival curve. Curve A is a "single hit" curve and curve B is a "multiple hit" (five hit) type. The method of determining the extrapolation number for curve B and the Do dose for each curve is illustrated by means of the dashed lines.

thing to notice about this graph is that the ordinate (the fraction of cells surviving) is a logarithmic scale whereas the abscissa (the dose) is a linear scale. The curve marked A is an exponential or "single hit" survival curve and gives a straight line survival curve on this semi-logarithmic plot. This is the type of survival curve to be expected from an organism with a single target. The curve marked B is a sigmoidal or "multiple hit" type of survival

curve which is characterized by the presence of the shoulder at low doses which may indicate that there are a number of sensitive targets within the single organism and each of these must be destroyed before the organism will die. If the curve is truly of the multiple target variety, then simple mathematical analysis indicates that the extrapolation number is equal to the number of sensitive targets. The extrapolation number, as its name implies, is the point where the extension of the linear portion of the survival curve intersects the zero dose axis. The fact that the extrapolation number may be equal to the number of targets explains the obvious interest in the extrapolation number since it may give an idea of the nature of the sensitive target merely from the number of such targets which exist within the organism. The other feature which is of prime interest in the analysis of survival curves is the slope of the line once it has become straight. As has been pointed out by Dr. Hunt, this slope is related to the volume of the target and a simple calculation will enable one to calculate a rough target volume from the slope of the survival curve. The fact that in Fig. 3 both curves have the same final slope indicates that in both cases the volumes of the individual targets are equal although in system B there are five identical targets per organism while in system A there is only one target per organism.

It is customary to designate the slope of the survival curves by a value known as the Do dose. This is the dose which will on the average put one hit into one target in each organism. The Do dose is easily determined since it is the dose required to reduce the fraction of survivors by 37 percent once the straight line portion of the survival curve has been reached.

It is customary to refer to the number of targets as the multiplicity of targets and this number is exactly equal to the "extrapolation number". If the simple target theory were the correct explanation for all radiation effects on biological systems, then the extrapolation number and the multiplicity of targets would be identical and one term would be redundant. Unfortunately, however, there are other effects which could give a curve a shoulder which would not necessarily mean that there were multiple targets in the biological system. It might merely mean that the radiation was producing toxic substances within the system which, when they reached sufficient concentration, could cause the death of the organism. Under these conditions the survival curve would have a shoulder, since at low doses there would not yet be a toxic concentration, but this shoulder would not be interpretable in terms of a number of sensitive targets.

All x-ray survival curves for mammalian cells, determined to date by means of *in vitro* techniques, appear to have at least a slight threshold. The presence of a threshold indicates that damage must accumulate within the cell before cell death can occur.

Survival curves for two lines of mammalian cells—the L cell, derived originally from mouse tissue, and a line of cells derived from Chinese Hamster lung, are shown in Fig. 4. The L cell has an extrapolation number of 2 and a Do

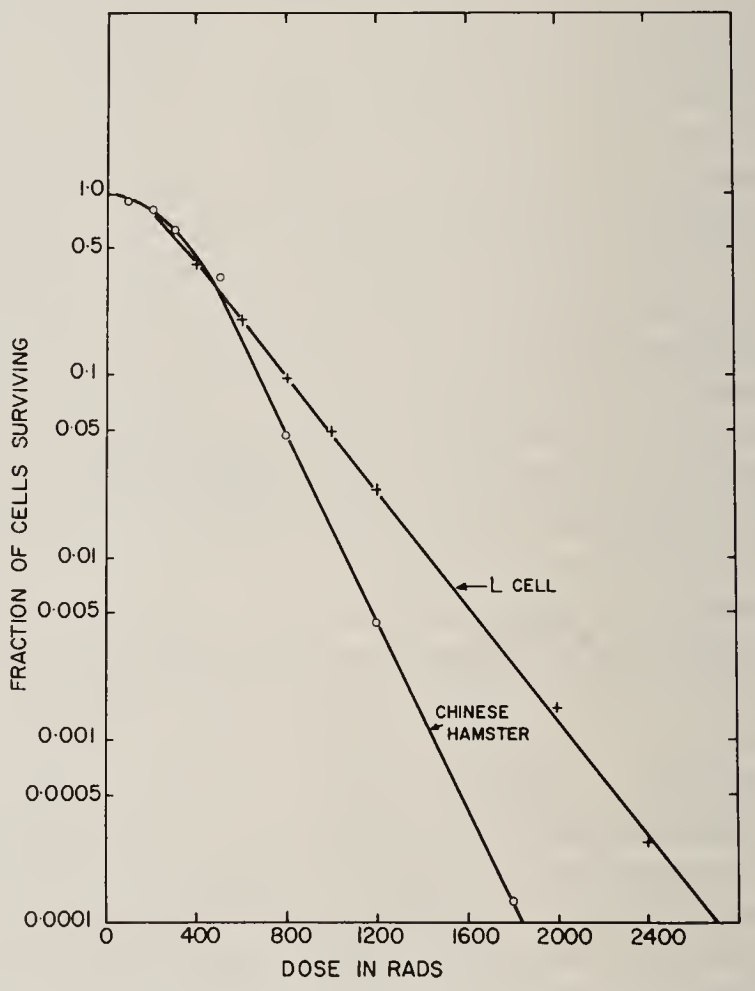


Fig. 4. Radiation survival curves for the L cell and a line of cells derived from lung tissue of the Chinese Hamster.

of 274 rads whereas the Hamster has an extrapolation number of 5 and a Do of 170 rads. It will be seen that the mammalian cells are all very sensitive to radiation having Do values of the order of 100 rads whereas the Do value of most bacteria is 5,000 rads and the doses required to inactivate most enzyme systems are of the order of $10^{-7}$ rads. This extreme sensitivity suggests that the radiation sensitive target within the cell is large since the radiation sensitivity is very roughly proportional to the physical volume of the target.

## THE ROLE OF CHROMOSOMES

The fact that the radiation target in mammalian cells is so large has suggested to many

investigators that the target might be the cell nucleus or more probably the chromosomes within the nucleus, and certain evidence has accumulated to support the latter theory. At the first mitosis following irradiation chromosome aberrations—breaks, rings, bridges etc. are observed. All of these phenomena tend to reduce the chance that after mitosis both daughter cells would have viable chromosome complements. Fig. 5 shows typical chromosome complements

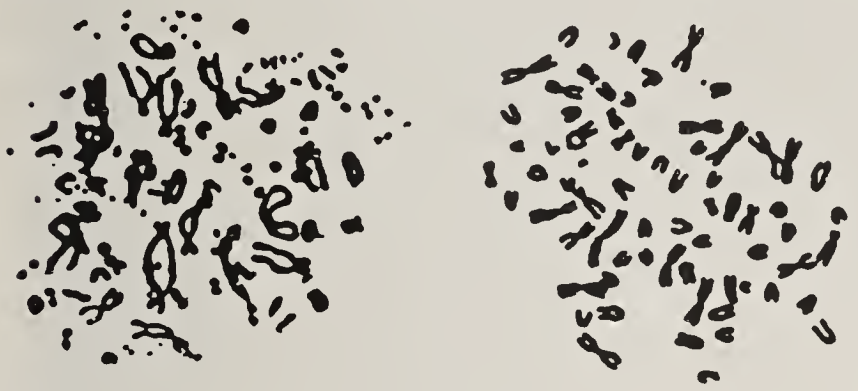

Fig. 5. The left half of the figure shows the normal L cell chromosome complement while the right half shows the appearance of the chromosomes at the first mitosis following a dose of 5000 r.

of cells before and after irradiation. The dose of radiation which on the average will cause one chromosome aberration per cell is roughly equivalent to the dose of radiation which will reduce the number of surviving cells to 37 percent, indicating that chromosome aberrations could indeed be the cause of radiation death.

However, Till (1) has recently carried out an experiment designed to determine the effect of chromosome number on the radiation sensitivity of various strains of L cells. The simplest hypothesis used to link chromosome damage with cell death from radiation requires that the sensitivity of a cell to radiation should increase with chromosome number since the chance for chromosome abnormalities resulting from radiation will increase with chromosome number. For his experiments, Till used L cell lines with chromosome complements of 53, 60 and 109 and found that all the cell lines had the same radiation sensitivity. This result requires that the simplest hypothesis relating chromosome aberration to cell death must be modified in a way which is not yet clear.

As has been pointed out, the fact that radiation survival curves for mammalian cells possess thresholds means that cells can accumulate sublethal damage from which they may recover, provided that such recovery occurs before the cell accumulates a lethal amount of damage. Elkind (2) at the U.S. National Institute of Health, has been able to show the existence of such recovery phenomena and has pointed out its obvious importance to radiotherapy. In radiotherapeutic practice it is usual to give a tumor a lethal dose spread over a period of several weeks. If cells are able to partially recover from radiation damage, then the chances of destroying a tumor will be a complex function of the radiation sensitivity of individual cells, the recovery rate of sublethal damage, the way in which the total dose is fractionated, the number of cells within the tumor and the number of cells required to permit growth of the tumor. In general, however, if recovery processes do occur, then a small dose is given in one or two treatments will be as effective in destroying a tumor as a larger dose spread over many days because in the single treatment there will be no time for recovery but in case of treatments spread over many weeks the cells will have opportunities to recover between every treatment. This general result is borne out by radiotherapeutic findings but the picture is clouded by other complications which will not be detailed here.

## COMPARISON OF RADIATION SENSITIVITIES *in vivo* AND *in vitro*

So far the data presented on cell survival have been concerned only with the survival of mammalian cells grown both before and after irradiation in tissue culture and it might be argued that the radiosensitivity of cells under these artificial conditions might bear little if any resemblance to their sensitivity in the *in vivo* situation. By means of an elegant experiment Till and McColloch (3) have been able to measure the *in vivo* sensitivity of mouse bone marrow cells and the values of extrapolation number and Do found for bone marrow cells irradiated either *in vivo* or *in vitro* in these experiments are in excellent agreement with the *in vitro* values for other mammalian cells. In another experiment Hewitt and Wilson (4) have measured the *in vivo* sensitivity of mouse leukaemia cells and again the results are in excellent agreement with *in vitro* results obtained for other cell lines. These results encourage the belief that it may be possible to draw valid conclusions about the sensitivity of cells *in vivo* from the results of *in vitro* experiments.

From the experimental results given so far, it is possible to draw a number of general conclusions. Mammalian cells are very sensitive to radiation and a dose of the order of 250 - 600 r will reduce the number of cells capable of continuous division to about 10 percent of its

original value. The variation in sensitivity for mammalian cells is probably over a factor of 2 which, while not being a large factor, could be of obvious importance to radiotherapists if tumor cells were always at one extreme or other. The shape of the radiation survival curves for single cells is always sigmoidal implying the presence of sublethal radiation damage which may be recovered from if the cell does not receive additional damage before recovery can take place. It appears that it is possible to extrapolate at least certain *in vitro* results to the *in vivo* case.

## EFFECTS OF RADIATION ON CELLULAR FUNCTIONS OTHER THAN DIVISION

In all of the experiments outlined so far we have been concerned with the ability of an irradiated cell to carry out the number of divisions required to give rise to a colony containing 1000 or more cells and the "survival" curves have been a measure of this ability. While the ability to divide may be a suitable measure of the survival of tumor cells, the less differentiated marrow cells, or the cells of any tissue in which there is a rapid cell turnover, it may not be a suitable criterion to use for other cells which, at least in the adult body, do not divide at all or perhaps only rarely and are more concerned with the production of hormones or the carrying out of other specific functions not necessarily related to cell division. In an attempt to see how cellular functions, other than division, are affected by radiation and also if possible to learn what constitutes a "hit" in such a cell, experiments have been carried out to observe the biochemistry of cells following massive doses of radiation.

## DNA SYNTHETIC CYCLE IN UNIRRADIATED CELLS

Before describing the results which were obtained following irradiation, perhaps it would be wise to describe some aspects of the normal cell cycle of an unirradiated cell growing in tissue culture. (5) Under favourable conditions cells in tissue culture will double their number approximately every twenty hours. This means that, on the average, each cell in the population will pass from one mitosis through the succeeding interphase and into the next mitosis every twenty hours. During this time each cell must synthesize enough DNA, RNA and protein to construct a new cell. Because of the repetitive nature of this process, it has become customary to speak of the life cycle of a single cell and attempts have been made to derive the time relationships for various processes inherent in the life cycle. These attempts have been most successful in the determination of the time sequence of DNA synthesis; the attempts to study RNA synthesis have been less successful because of the greater complexity of the problem and with protein, the work has scarcely been started. The method by which the DNA cycle has been worked out will be described not only because DNA, the material of chromosomes, is such an important part of the cellular substance and has been postulated to play a major role in determining radiation damage, but also because these experiments indicate the techniques and modes of experimentation which are becoming common to cellular radiobiology today.

The one feature of the cell cycle which can be clearly recognized is mitosis, the period when the chromosomes become visible and during which the two identical parts of each chromosome separate and take up their position in daughter cells. Because of its ease of recognition, mitosis is taken as the reference point in any determination of cell cycles. The total duration of the average cell cycle is equal to the time taken for the number of cells in the population to double and this is an easily determined quantity.

In a rapidly growing cell population cells exist at any given time at various stages in the cell cycle, some are in mitosis, some are in interphase (Fig. 7). The fraction of cells which are in mitosis at any time is approximately equal to the fraction of the total cell lifetime which is occupied by passage through mitosis. For the L cell this fraction is about 2.5 percent indicating that mitosis occupies about 2.5 percent of 20 hrs. or 30 minutes. In much the same way we can determine the fraction of the cell cycle which is required for DNA synthesis. To do this we add tritium labelled thymidine, a radioactive precursor of DNA. When the thymidine is added, those cells which are synthesizing DNA at the time of addition will immediately take up the radioactive precursor and will themselves become radioactive. If a sample of cells is removed from the growth medium shortly after the radioactivity is added it is possible to detect the fraction of cells which are radioactive by the technique of radioautography. In this procedure the cells, a fraction of which contain radioactivity, are stained, allowed to attach to a glass plate and are then (in the dark) overlaid with photographic emulsion and kept, in the dark, for a period of several weeks. After this time the slides are developed. Underneath the developed

emulsion, each cell is clearly visible. Directly above each cell which contains radioactivity, the photographic emulsion is affected and when developed will reveal a large number of black dots, due to the passage of electrons through the emulsion, Fig. 6.

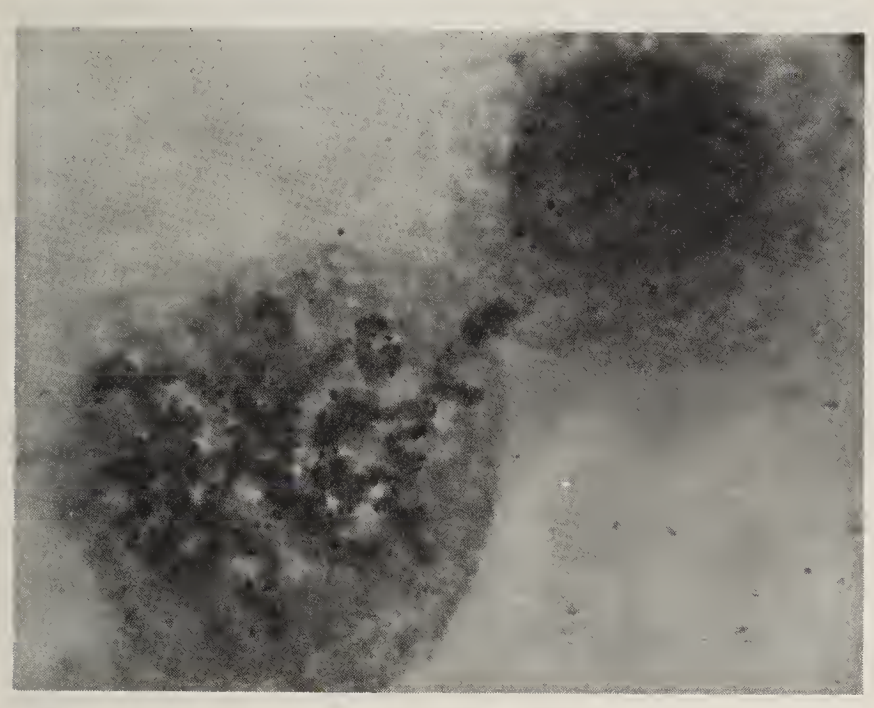

Fig. 6. Radioautographs of two cells, one in interphase, the other in mitosis. Note that the exposed photographic grains are localized over the nucleus in the interphase nucleus and lie almost directly over the chromosomes in the mitotic figure.

It is a simple matter to determine the fraction of cells which contain radioactivity and this fraction is very nearly equal to the fraction of the life cycle which is concerned with DNA synthesis. For the L cell the fraction of cells which take up label immediately on addition is 0.30 indicating that DNA synthesis requires 0.30 x 20 = 6 hours.

The fact that DNA synthesis requires 6 hours still does not tell us when this synthesis occurs with respect to mitosis. However, by combining the methods of autoradiography with the search for mitotic figures, it is possible to determine the amount of time which elapses between the completion of DNA synthesis and mitosis. This is accomplished by adding tritium labelled thymidine to a culture of cells, removing samples at various times and preparing autoradiographs as described above. On these slides, however, one does not look merely for the presence of radioactive cells but one looks for the presence of radioactive mitotic figures, Fig. 6. The time which elapses between the addition of the radioactivity and the appearance of the first radioactive mitotic figure represents the time between the end of the DNA synthetic period and the beginning of mitosis. This period between DNA synthesis and mitosis is known as the G2 or postduplication period and is 3 hours in duration. We have now seen that mitosis occupies 0.5 hours, DNA synthesis 6 hours, G2 3 hours and therefore the time between mitosis and DNA synthesis, the G1 or preduplication period, must equal 20 - 0.5 - 6 - 3 = 10.5 hours. This then completes a determination of the DNA life cycle for L cells and the results are depicted pictorially in Fig. 7.

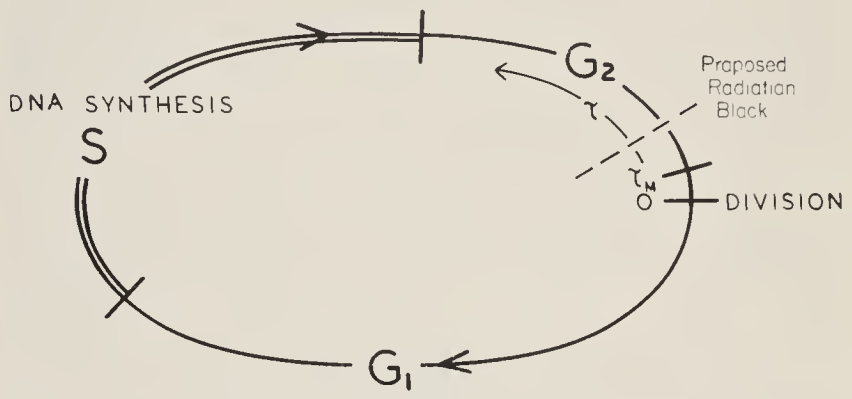


Fig. 7. Schematic representation of the DNA synthetic cycle, showing the time sequence of mitosis, G1, S and G2.

Results obtained with other cells show similar DNA cycles with slight variations in timing from those presented here.

## EFFECT OF RADIATION ON THE CELL CYCLE

Having established the foregoing it becomes possible to observe the effect of radiation on such a culture with the hope that some clue as to the type of damage which has heretofore been described as a "lethal hit" will be obtained.

In the experiments which follow, the doses of radiation employed were so large that no surviving cells remain; the fraction of cells capable of continuous division after the irradiation being less than 0.001, i.e. less than one cell in a thousand has escaped being "hit".

In discussing the effect of radiation on various parts of the division cycle, it may perhaps be best to advance a rather simple model and then show the data which supports this model. The proposed model is that the principal effect of radiation is to induce a block in the cell cycle immediately prior to mitosis (Fig. 7). Initially this block prevents cells from leaving G2 and entering the mitotic period but in no way interferes with the passage of cells through other parts of the cycle - i.e. cells flow from G1 into S and from S into G2 at the normal rate but here they are stopped. The evidence to be presented also seems to indicate that for any one cell this block may or may not be overcome because of recovery processes acting within the

cell. If the block is not overcome the cell cannot give rise to offspring, but even if the block is overcome the cell may or may not give rise to viable offspring. In order to test the validity of the model, we would like to observe cells as they pass from one period to another and, as they perform various functions, to see which parts are interfered with and at what time after irradiation. Some of the features which we can observe are mitotic frequency, the passage of cells from G1 into S, the synthesis of DNA by cells in S, the rate of flow of cells from S into G2 and the reappearance of any or all of these features after the block is recovered.

If the proposed radiation block occurs immediately before mitosis, there should be following irradiation an immediate cessation of mitotic activity, with the mitotic frequency in the population dropping to zero and remaining at this level until the radiation-induced block is overcome, whereupon the mitotic index should rise to some unpredictable level. Fig. 8 shows the mitotic index as a function of time in

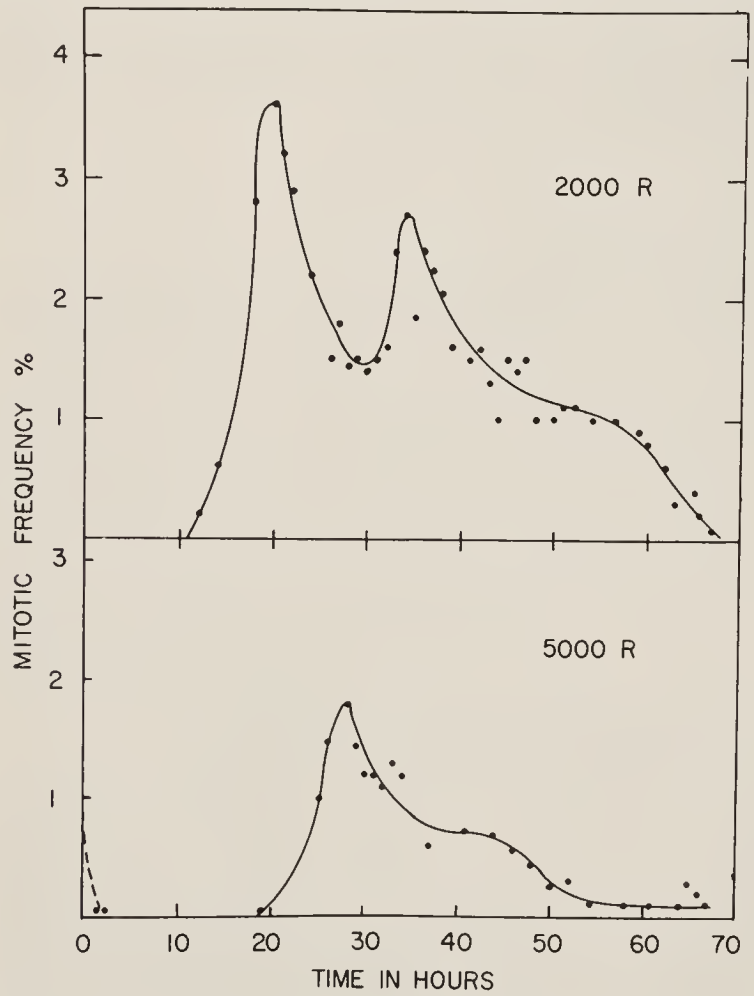


Fig. 8. Mitotic frequency as a function of time in cultures which have received 2000 and 5000 r. The average mitotic frequency in unirradiated cultures is 2.5 percent.

populations of cells given 2000 and 5000 r. Immediately following irradiation with 2000 r the mitotic frequency does drop to an almost negligible value but 13 hours later mitotic figures reappear in the culture. After 21 hours mitotic figures begin to reappear in a culture given 5000 r. In the culture given 2000 r the mitotic index actually rises somewhat above the normal value.

If, after irradiation, the mitotic index of a cell population drops to zero, there should be no increase in cell number until such time as mitotic figures reappear when there may or may not be an increase depending on whether or not the mitosis that is observed terminates in the formation of daughter cells. The presence of a mitotic figure does not itself guarantee that the cell will be able to complete a division. If the chromosomes have been too badly damaged by the radiation the cell may not be able to organize the chromosomes for a successful nuclear division and instead of forming two identical nuclei the cell may be left with a single giant nucleus containing all the chromosomal material or several micro nuclei containing only fractions of the total chromosome material. Even if the cell does apparently carry out a successful nuclear division and subsequent cytoplasmic division, either one or both of the daughter cells may be non-viable because they lack parts of, or whole chromosomes, or because of radiation induced mutations within the chromosomes. Fig. 9 shows the increase in cell number in a

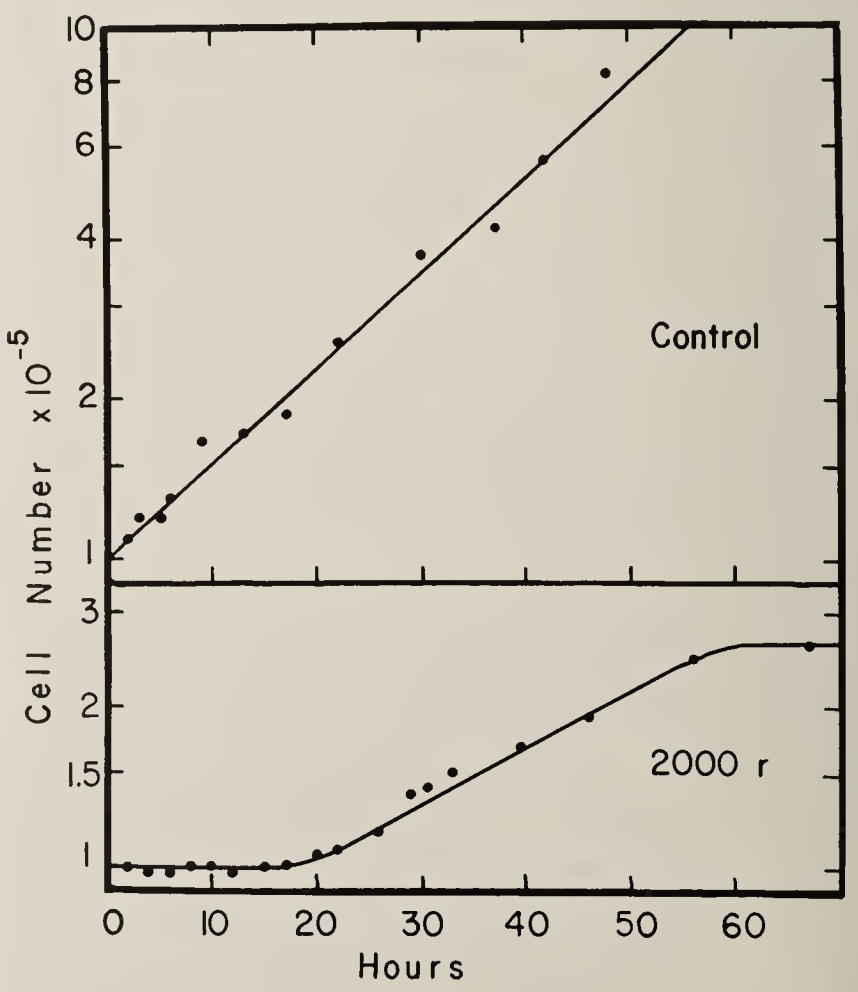


Fig. 9. Growth curves of an unirradiated cell population and of a population given 2000 r.

control culture and a population of cells given 2000 r. After a dose of 2000 r the cell number becomes somewhat more than doubled before division ceases. After 5000 r division is delayed even longer but about 10% of the cells divide at least once before division ceases. The fact that after 5000 r, 10% of the cells divide once does not indicate that these 10% are capable of continuous cell division because Fig. 4 indicates that after a dose of 5000 r only a negligibly small fraction of the cells are capable of continuous division. Most of the cells in the 10% divide once or at most a few times and give rise to "abortive clones". The formation of these abortive clones may partially explain why some tumors appear to grow somewhat after radiotherapy has begun.

In continuing to observe the progress of cells around the division cycle following irradiation, the next observation point is the place where cells pass from G1 into S. Tritiated thymidine, the DNA precursor mentioned earlier, can be used to measure the rate at which cells pass from G1 into S, since each cell as it passes from G1 into S will begin to pick up radio-active label and if the number of cells which pick up radioactivity is determined as a function of time, the rate at which cells are passing from G1 into S can be established. Using the single block model we should expect that following irradiation the curve indicating the percentage of cells that are labelled will follow the control curve until the last cell to escape the block has passed into S and then no cells should enter S until such time as the block is removed and the cells which were behind the block have had time to pass from G2, mitosis, G1 and into S. This should occur about ten hours after the reappearance of mitotic figures. Since about 20% of the cells were in G2 at the time of irradiation and since these would be unable to take up label until the block reversed, we should expect that the initial level portion of the curve would occur with about 80% of the cells labelled.

In an experiment the rate of flow of cells from G1 into S following a dose of 2000 r was determined and the results are shown in Fig. 10. It is seen that following even this large dose, the rate of flow of cells into the S period is practically unhindered until such time as the block in G2 makes its presence felt, and when this occurs flow into S ceases. At this time about 80% of the cells are labelled; this is in agreement with the assumption that 20% of the cells are trapped between the end of DNA synthesis and the radiation induced block and are therefore incapable of picking up label. However,

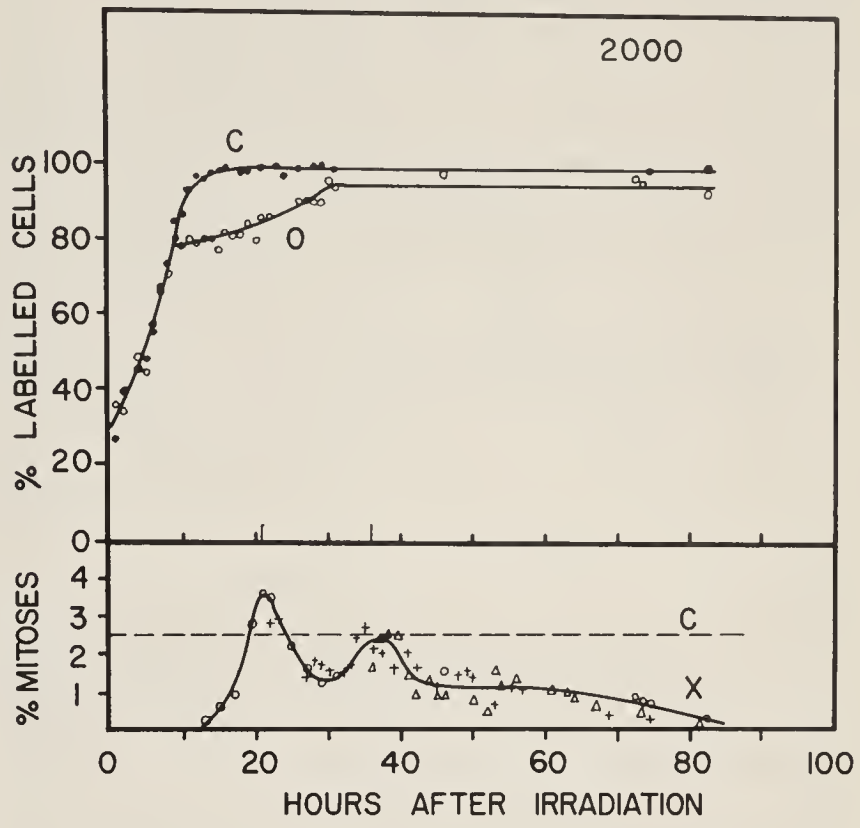


Fig. 10. Illustrating the rate of increase in the number of cells which have taken up tritium thymidine as a function of time in a control culture (c) and a culture given 2000 r (0).

after the block has been removed, these trapped cells may be able to pass through mitosis and G1 and into S and this will account for the secondary rise in the percent of labelled cells from 80% to 95% which occurs about 10 hours after mitotic figures have reappeared in the culture.

Up until now it has been possible to observe the progress of cells at mitosis, at division and passing from G1 into S. Finally, it is possible to measure the rate of DNA synthesis while the cells are in S and thus complete the observation of radiation damage throughout the cell cycle. On the basis of the single block model, the total DNA in an irradiated culture should increase at the same rate as in a control culture for a time equal to G1. At this time no new cells will be passing from G1 into S but some cells will still be in S so DNA synthesis will proceed at a reduced rate until a time equal to G1+S when it will cease entirely because there are now no cells in S. However, about ten hours after the block is removed and mitotic figures reappear in the culture, DNA synthesis should recommence. Fig. 11 shows the DNA synthesis as a function of time in a culture which has received 2000 r and also an unirradiated control

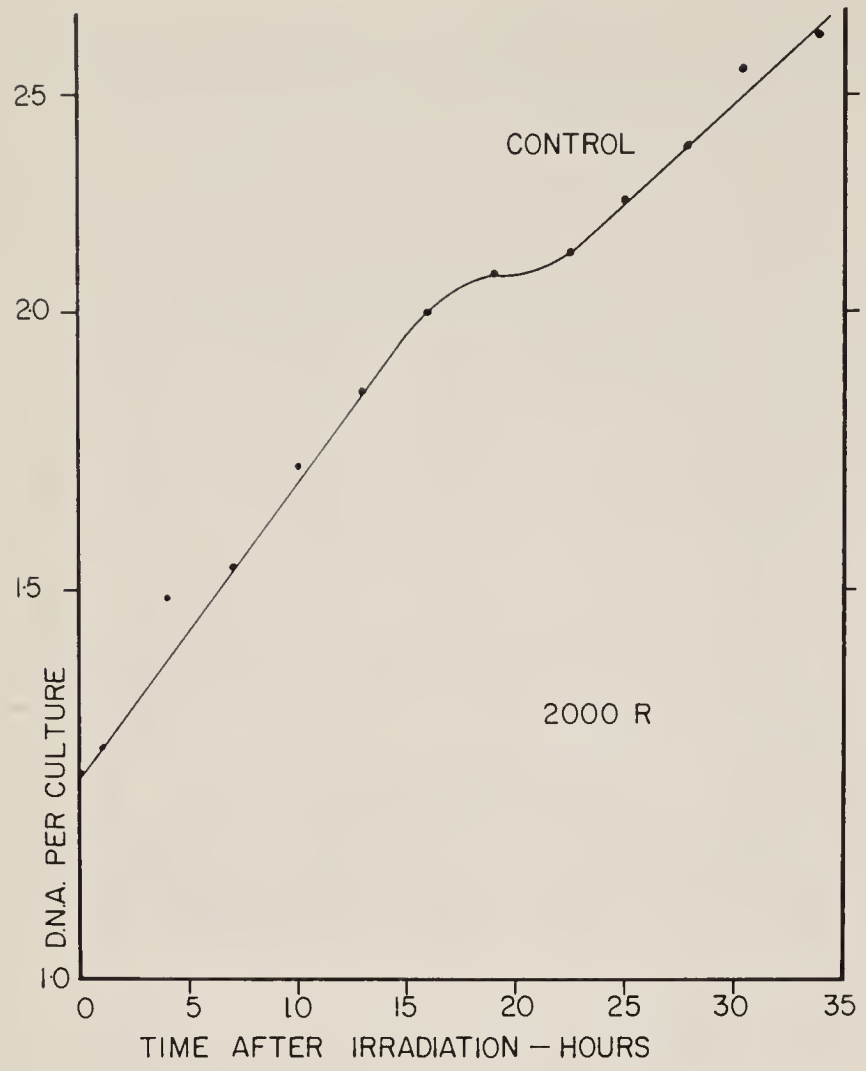


Fig. 11. DNA synthesis as a function of time in a culture which has received 2000 r.

culture. After a dose of 2000 r, the rate of accumulation of DNA during the first hours after irradiation is almost identical to that of the control. There is then a brief period when no synthesis occurs in the irradiated culture and this is followed by a period of increased synthesis at a time about 10 hours after the reappearance of mitotic figures.

Finally, Fig. 12 shows the DNA synthesized per cell over very long times after irradiation;

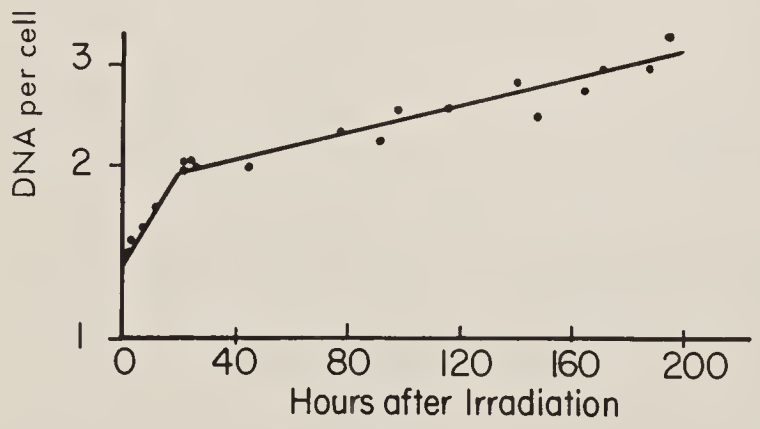


Fig. 12. Accumulation of DNA over long periods of time after irradiation. Note that after irradiation cells may accululate more than 3 times their normal complement of DNA.

this shows that DNA synthesis does go on for very long times and because there is no cell division, the value of DNA per cell will reach 3 or 4 times normal. These cells which contain abnormally large amounts of DNA and other cellular constituents are referred to as giant cells and their formation characteristically follows large doses of radiation.

The experimental evidence presented above seems to indicate conclusively that, whereas radiation damages cells no matter what phase of the cell cycle they are in the principal effect of radiation is manifested when the cells enter the phase where their further progress through the cycle is blocked. Most of the observed effects of radiation in producing mitotic delay, division delay, reduced DNA synthesis, etc. during the first post-irradiation cycle can all be explained by the presence of this block. Finally, however, this block may sometimes be overcome with cells again entering mitosis. Even after a dose of 5000 r, 15% of the cells divide at least once. These cells are not radiation survivors in the sense that they escape a hit or hits because at this dose the number of survivors is probably less than one in $10^8$. The cells which escape the block have at least two possible paths open to them; they may carry out one or two abortive divisions before division ceases completely, or they may somehow circumvent the division step and go on to form giant cells.

In conclusion it might be wise to summarize some of the positive findings of experiments such as these and then to point out a few of the places where much more detailed knowledge is required.

The data presented here are in agreement with the initial assumption that the ability of cells to carry out continuous divisions is easily destroyed by radiation. For almost any cell, a dose of 500 r will reduce the fraction of surviving cells to somewhere between 1% and 10%. There are in the cell many enzymatic reactions such as the synthesis of DNA, RNA and protein which are very insensitive to radiation and therefore it is unlikely that the cause of radiation death is a gross lack of synthesis of any of these three constituents. There is, however, at least one part of the cell cycle which is extremely sensitive to radiation damage and this manifests itself as a block in the G2 region of the division cycle. It must be emphasized however that this does not mean that the cells which are in G2 are necessarily more sensitive to radiation. It merely means that wherever cells are in the cycle at the time of irradiation they suffer damage

but this damage is not evident until the cell reaches G2. The nature of the reactions involved in this block are still a mystery which appears to await the development of new and refined techniques of biochemistry or biophysics before it will be solved.

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## BOOK REVIEWS

Pathology, a Dynamic Introduction to Medicine and Surgery; T.M. Perry and F.N. Miller, Jr.; Little, Brown and Co., 598 pages, $5.50.

Written primarily for the medical student by Professor Peery and his Associate, Miller, of the George Washington University School of Medicine, this book might be passed by as just another of the many texts of pathology. As a compromise between the large, expensive reference works and the brief, often useless "aids", this book is no better or worse than its competitors with respect to the material included and the presentation of same, the latter being dynamic and frequently personalized (e.g., "Please do not make the error of saying,'There is no evidence of pathology in the lungs.' Pathology does not mean abnormality. Repeated misuse has given this meaning of the word a certain degree of acceptance, but it is still objectionable, especially to a pathologist!). The worst fault is the lack of any illustrations. However, there are some very positive things to be said for this work.

Every key word in the text is underlined and the margin of each page contains the organizational headings beside the appropriate paragraphs so that the result is similar to a magnificent set of notes, the kind one likes to borrow. This is invaluable when the student's mind drifts to next Saturday's date and is recalled by the thought of tomorrow's examination; a quick glance up the margin of the page he is staring at will renew the subject matter covered enabling him to carry on without reading the whole page again for orientation. A short review of the margins would also serve to consolidate what the student has read. The underlining is done in precise fashion so that just reading these words will unlock the mysteries of a whole page of print. These two features make this text a useful review for one who has finished his formal indoctrination, viz., senior medical students, residents, etc., but needs a fast "cram" before sitting an examination requiring a knowledge of pathology.

*Pathology* is a fine example of the "paper-back, throw-away" textbook. This editor advocates these books, which can be bought, used and discarded very cheaply, for the student market. The use of them abolishes the weeping one does when he finds that all the Morocco-bound tomes in his library have been outdated in 4 short years.

L.E.S.

●

Aids to Forensic Medicine and Toxicology; Sir W. Bently Purchase; Macmillan Co., Can., 254 pages, $ 2.15.

The thirteenth edition of this text has been entirely re-written because of the ever widening scope of forensic medicine. The object is to provide a knowledge sufficient to enable the student and average doctor to appreciate the impact of the law and social custom on medical practice.

The text is divided into 3 parts: part one deals with the law in relation to medicine and dentistry; part two treats the practical aspects of death; a fairly comprehensive synopsis of toxicology is the subject matter of the third part.

This small volume would be a good place to start for those who want to find out about the field of legal medicine.

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# DYNAMICS OF RADIATION PATHOLOGY

W.R. BRUCE, M.D., Ph.D.

The primary action of radiation on tissue can not be seen and its nature remains obscure, but cells are injured and the results of their injury are subsequently manifested by altered cell and tissue function. The observed pathological picture may be difficult to interpret because the chain of events from the initial injury to the final change may be long and involved. However by unravelling the consequences of consequences it is possible to explain the observed lesions as the final result of a primary effect of radiation. For example, some days following total body radiation an animal may display petechiae and hemorrhages. Examination of the blood reveals thrombocytopenia; examination of the marrow, a scarcity of megakaryocytes, and further examination will show that this is in turn secondary to a marked depletion in the number of stem cells that make megakaryocytes. Radiation has prevented the stem cells which are normally actively multiplying from proliferating. This primary effect of radiation becomes apparent as hemorrhage days later.

In addition to the radiation injury that blocks the multiplication of cells, investigation has revealed two other primary effects of radiation. The second is due to damage of the macromolecules of the cells' nuclei which gives rise to cell mutations; these may occur in either somatic cells or in the germ cell series. The third is cytoplasmic damage which leads to malfunction or death of the cell. The relative importance of these three primary radiation injuries varies with the dose of x- or gamma-rays. Genetic effects (mutations in cells of the germ cell series) are the most important at low doses of radiation. The action of radiation on inhibiting the proliferation of cells is of greatest importance in dosages from a few hundred to thousands of rads. The direct lethal action of radiation is most important at very large doses of the order of thousands of rads—larger than usually encountered clinically.

At any dosage all three effects operate but at any given dose one of them is usually by far the most important. We will trace the results of each of the three radiation effects in turn and then consider their relative importance following whole body radiation. Our attention will be directed largely toward the action of radiation on proliferating cells for it has great significance in radiopathology.

## ACTION OF RADIATION ON PROLIFERATING CELLS

For many years it has been stated that tissues composed of rapidly multiplying cells are more "sensitive" to radiation than those that are not (1). Bone marrow shows a much greater response to radiation than muscle. Bone marrow also exhibits many many times more mitotic figures than muscle tissue. However, at present we have much more than a qualitative correlation between mitotic activity and "radiosensitivity", for many quantitative studies have established the importance of cell multiplication in the display of radiation damage.

Tissue culture studies, described by Dr. Whitmore, have added greatly to our understanding of the response of cells and tissues to radiation. They show in a quantitative way the degree to which radiation reduces the ability of animal cells to multiply. This is predominantly an all or none phenomenon. Most of the cells are either damaged or "hit" so that they can no longer multiply or they are not. The number that escape damage decreases as the dose increases in the manner illustrated in Fig. 1 where the dose in rads is plotted on the X axis and the fraction of cells that can multiply is plotted on the Y axis. On this one graph are plotted data for non-malignant and malignant cells, for cells irradiated *in vivo* and *in vitro*. In all cases the radiation sensitivity is remarkably similar. 300 r prevents all but 10 percent from multiplying, 600 r all but 1 percent. The few remaining cells are able to continue to form colonies but those that have been hit run into difficulty when attempting to divide. Some fail to divide but continue to grow and form giant cells, others die, and, in tissues, many differentiate and do not divide any further. In recent radiobiology tissue culture literature it has become common to refer to cells that cannot divide as "dead" though of course many may still continue to function as giant or differentiated cells.

To what degree do these results of cell survival in tissue culture bear resemblance to cells in tissue? In tissue culture experiments the cells are multiplying at a high rate—about one division

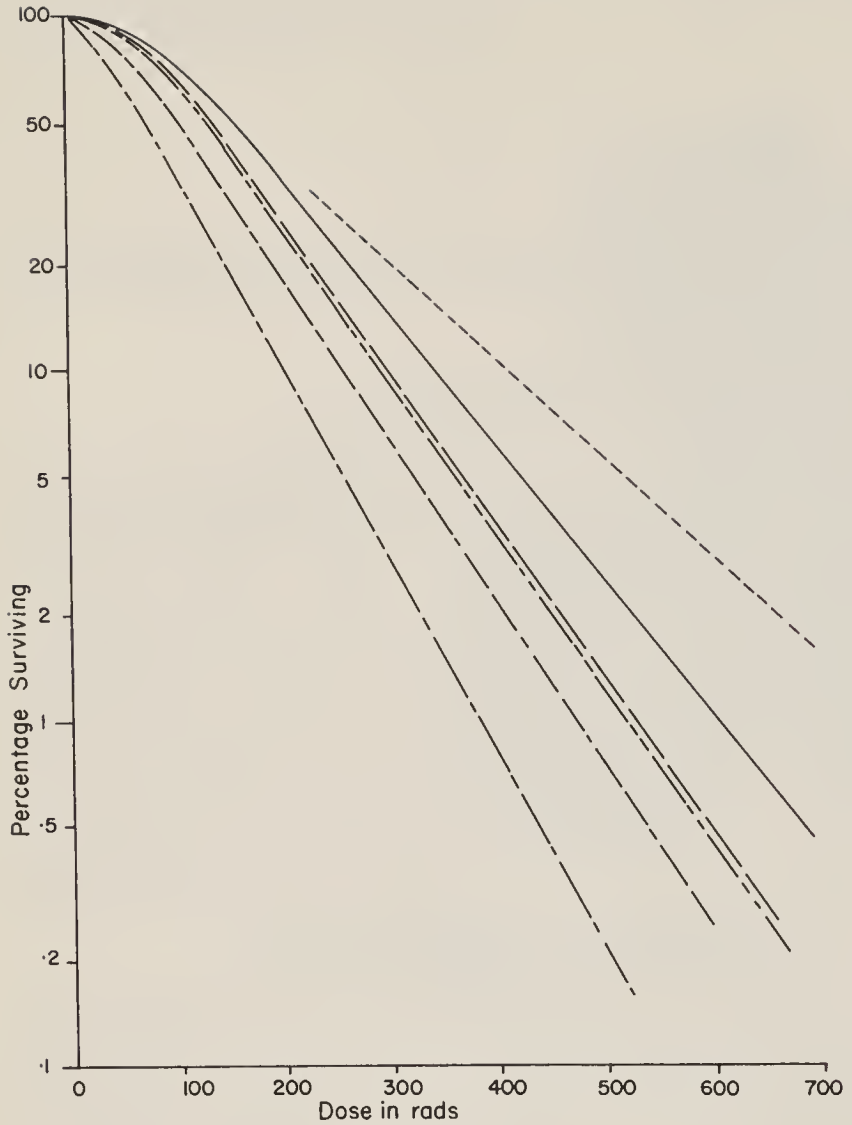



Fig. 1. The percentage of cells that are capable of continuous multiplication in tissue culture after vary-doses of radiation.

—— – —— Normal human epithelial cells irradiated in vitro (3)
—— —— Normal human fibroblast cells irradiated in vitro (3)
—— – —— Hela cells (human carcinoma of the cervix) irradiated in vitro (2)
—— – —— Mouse bone marrow cells irradiated in vivo (13)
——— Mouse bone marrow cells irradiated in vitro (4)
– – – – Mouse leukaemia cells irradiated in vitro (5)

This wide range of cells all have remarkably similar radiation sensitivities.

every 24 hours. Most of the cells in the body are multiplying much less quickly. They remain instead in the resting phase (G1) and only after some unknown stimulus begin to synthesize DNA and go through mitosis. All these cells are sensitive to radiation but, as was observed with cells in tissue culture, the injury is not apparent until they attempt to divide.

The latent sensitivity of irradiated cells is well demonstrated in the response of the liver (6). Normally there is a low mitotic rate and there is little visible effect of even large doses of radiation on this organ. But if a portion of the irradiated liver is removed so that the remaining liver cells are stimulated to divide the evidence of previous irradiation becomes apparent. Abnormal mitosis and giant cells appear. The injury received by the resting cell is latent until the cell reaches mitosis—exactly as was the case for cells in tissue culture.

The importance of cell multiplication in the demonstration of the radiation injury explains at once the apparent contradiction between the similar radiosensitivity of all cells in tissue culture and the range of tissue radiosensitivity seen in animals. The difference is due to the enormous range of cell replacement in different tissues. For instance, mitosis is rare and cell turnover negligible in muscle and brain tissue. As a result radiation damage to the nuclear structure is of no apparent handicap and no lesion is seen even after large doses of radiation.

At the other extreme of cell turnover and tissue radiosensitivity is the lining of the gastro-intestinal tract. Here there is a rapid extrusion and replacement of cells. Until recently its actual rate could only be estimated by mitotic indices after blocking with colchicine but the recent use of tritiated thymidine which labels newly formed DNA makes it possible to trace cells quantitatively from their proliferation to death.

The only epithelial cells in the small intestine that are initially labeled with tritiated thymidine are those at the base of the crypts (7). They are the only ones that are actively dividing and they display a cell cycle similar to cells in tissue culture with a generation time of about 18 hours. In the intestinal epithelium, however, one of the two cells produced in the mitosis normally does not pass through G1 but begins to differentiate into a mature columnar epithelial cell as shown in Fig. 2. As it does so it is pushed up the crypt and onto the villus by cells produced later by the germinal cells at the base of the crypts. The mature cell normally takes about three days to travel from the crypt to the tip of the villus where it is lost into the intestinal contents.

The predominant effect of radiation on the intestine is on the rapidly dividing cells in the crypts. The mature cells simply keep on moving out and into the intestinal lumen and are replaced in two to three days by cells formed following the radiation. Many of the cells irradiated in the cell cycle divide in mitosis and form two differentiating cells leaving no further source of new cells. Others continue to pass through the cycle without actually dividing at mitosis and become hyperploid giant cells. Others die. The end result is the depopulation of the intestinal epithelium starting at about three days after radiation. We will consider the clinical consequences in a later section.

The turnover of cells in the bone marrow is also rapid but more complex. The central pool

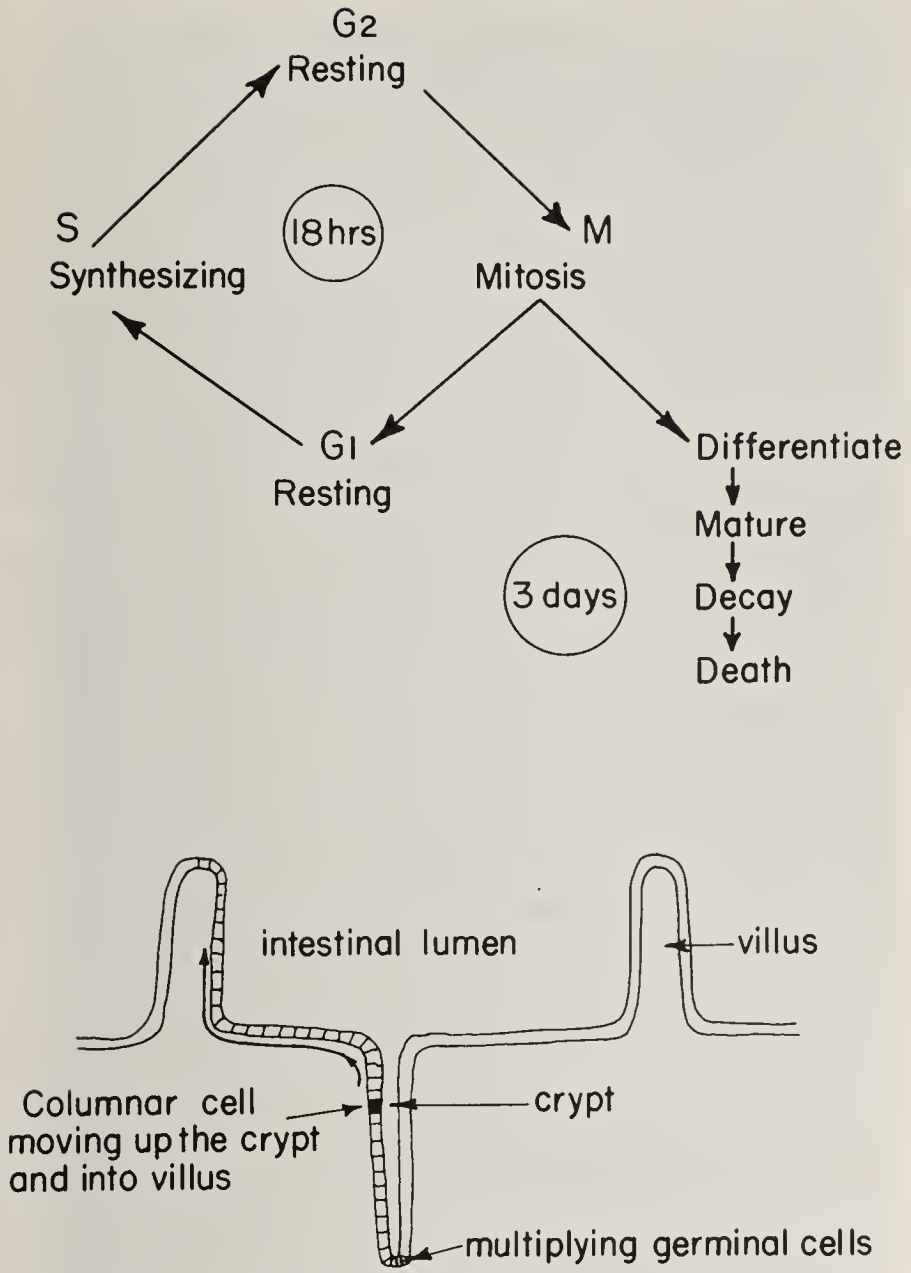


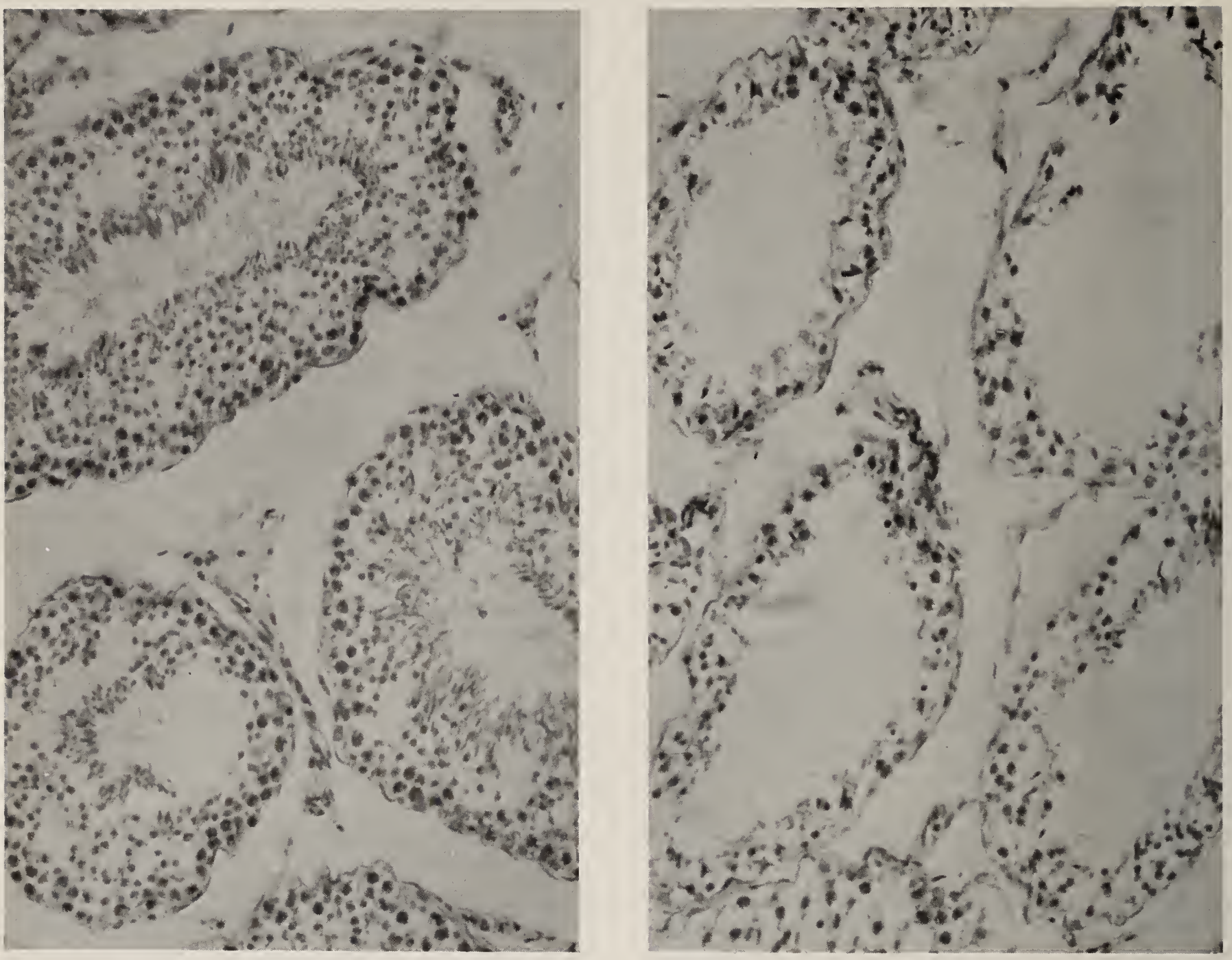

Fig. 2. The present concept of cellular repopulation in the intestine. The cell cycle occurring at the base of the crypts gives rise to cells which migrate up and onto the villi where they are lost.

of stem cells multiply with a generation time of about 24 hours (8). One of the daughter cells formed at each division continues as a stem cell. The other divides and differentiates to form members of the erythroid, myeloid or megakaryocyte series. For each division of the stem cell, perhaps 16 mature elements are formed. The lifetime of each from the initial storage in the bone marrow to the final destruction varies. In man, the red blood cells survive 120 days, the granulocytes perhaps 2 days. While platelets have a survival of about 4 days, their precursors, the megakaryocytes, remain in the marrow for a longer time. Following radiation to the bone marrow the sequence of events from stem cell to mature cell is broken. As was the case with the germinal cells of the intestine, the stem cells show the greatest damage. Many of them fail to go through mitosis, and die. Although their failure to produce new members of the dividing series follows within a relatively short period after irradiation, the effects of the radiation are not apparent for a period of days. The reason is of course that the animal continues to have the benefit of mature cells that are not dividing and are thus much less sensitive to radiation. When these are depleted it depends on the remaining stem cells to supply new members to the myeloid, erythroid and megakaryocyte series. Following whole body radiation there may be too few of these cells left to supply the need and the animal dies with leukopenia, anemia and thrombocytopenia.

The effect of radiation on the immunological response appears to be also due to the suppression of cell multiplication, for the period of greatest radiation sensitivity precedes that of greatest proliferation. Antibody production may be effectively reduced if the animal receives total body radiation at the time of its first exposure to the antigen (9). The primary response in which clones of cells capable of producing antibody are formed is suppressed. The secondary response is not reduced to the same degree for cells capable of producing antibody have already matured and hence the production of antibody after the primary response has occurred does not depend on the proliferation of new cells.

The action of radiation on the gonads again illustrates the importance of the action of radiation on proliferating tissues. Following a dose of several hundred rads many of the germinal cells, the spermatogonia, are unable to divide successfully so they die (10). After a period of days the spermatocytes, spermatids and spermatozoa become depleted and the seminiferous tubules appear to contain only the resting Sertoli cells (Fig. 3). The interstitial cells which also divide infrequently are little affected by the radiation and androgen continues to be produced. The male is thus potent but sterile. If the dose is not large the seminiferous tubules become repopulated with spermatogonia and in a few weeks the male's fertility returns. This is not to say that the sperm formed are unaffected by the radiation; we will consider the genetic effects later.

The result of local radiation to the female gonads is different. It prevents the cell division necessary for the maturation and development of primary follicles (Fig. 4) and leads to sterility as it did with the male. In the female, however, estrogen is largely produced by the cells of the follicle so that the female suffers not only cessation of menstrual periods but also a premature menopause. In the mouse there is still a further



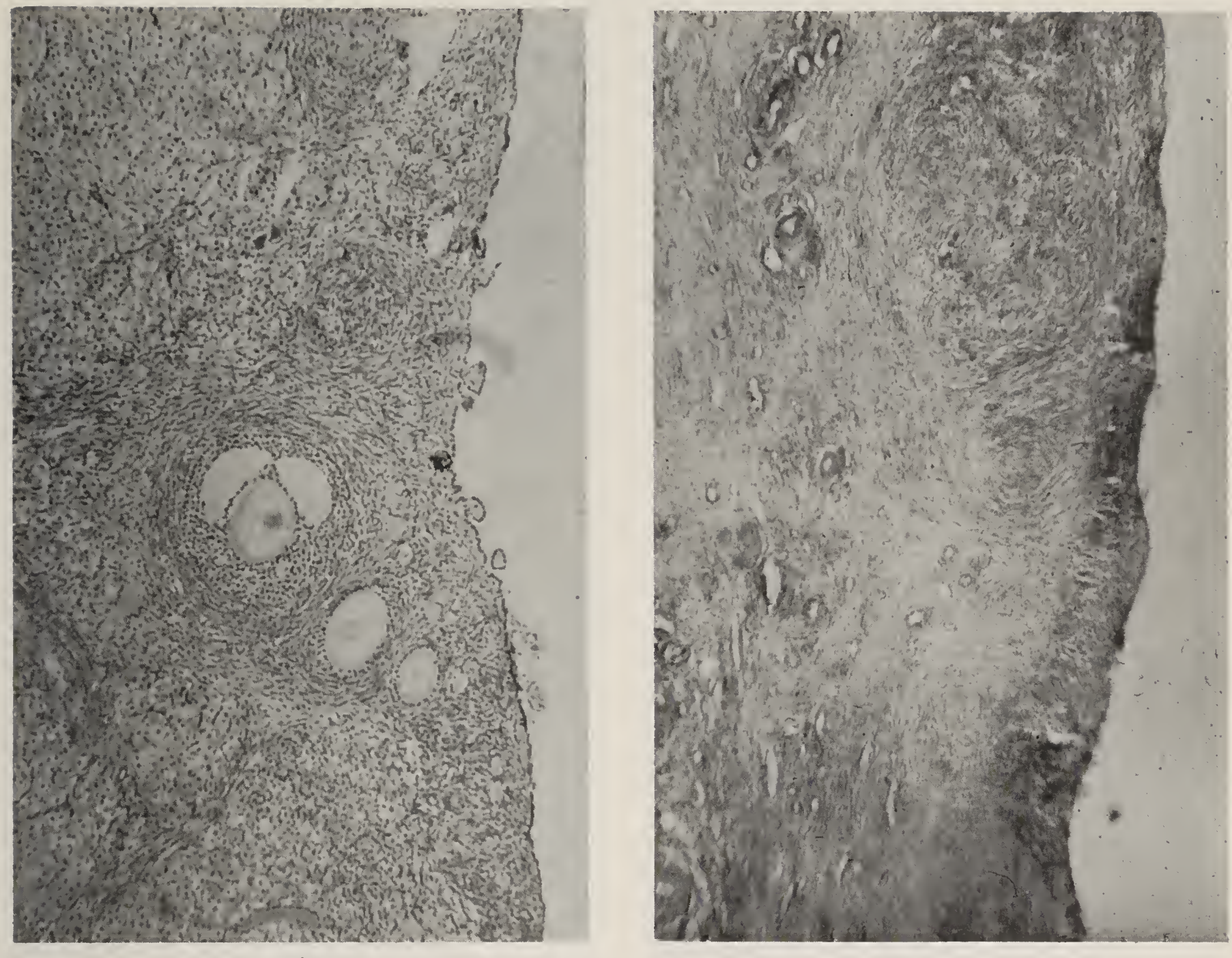
A B

Fig. 3. The testis of a normal rabbit (a) is compared to that of one 7 days after 1000 r local irradiation. Note that spermatogonia and spermatocytes are diminished in numbers while the Sertoli and interstitial cells persist. (H and E x 100).

consequence to irradiation of the ovaries. This is the occurrence of ovarian tumors. They are at least partially the result of pituitary hormone stimulation of ovarian cells by F.S.H. which is secreted in abundance because its secretion is not suppressed by estrogen in the blood stream.

The response of skin to irradiation is complicated by the behaviour of its blood supply. Immediately following radiation there is erythema. This is due to the release of histamine or histamine-like compounds and it disappears rapidly. The major response occurs in one to two weeks. Depending on the dose it may be observed as erythema, dry or exudative desquamation or as frank ulceration. The delay in the skin's response is again the result of the inability of germinal cells to multiply and maintain a normal, intact skin surface. Instead these cells either die or form giant cells so that for a period the dermis may be covered by only a single layer of these cells (Fig. 5). After irradiation the skin may be repopulated by the few cells that have fortuitously not been hit by the radiation or by cells outside the x-ray beam. Even years after the radiation the skin is thin and the appendages absent. The irradiated blood vessels are not able to form capillary buds normally and the irradiated skin is therefore very sensitive to trauma for without blood vessels granulation tissue cannot be formed.

The effects of x-rays on the skin contrast strongly with that of heat burns. The response to heat is much more rapid. The cells die immediately and not at the time of the next division. The damage from heat is usually down to some rather definite depth in the dermis. While all the cells of the epidermis are destroyed, cells of the deeper parts of hair follicles and sweat glands



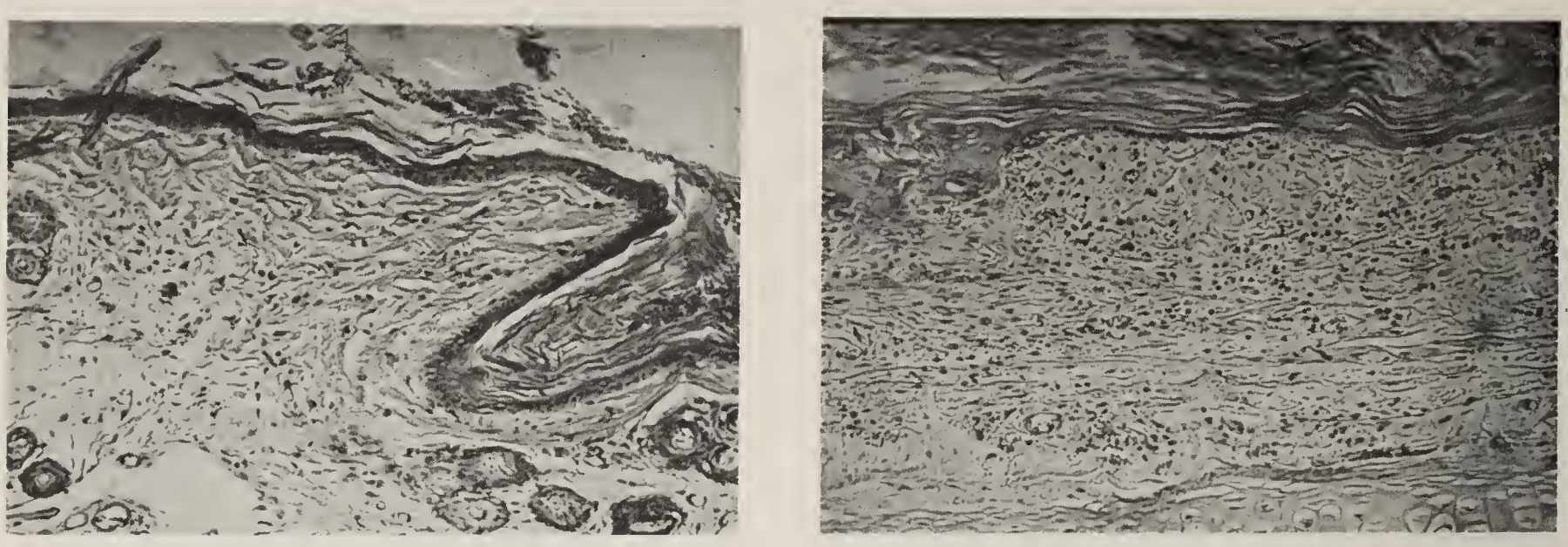
A B

Fig. 4. Normal ovary (a) is compared to that of a woman who a year previously had received 2000 rads to the abdomen. Note the complete absence of follicles in the irradiated specimen. (H and E x 35).

may persist and they repopulate the epidermis. In x-irradiation both the epidermis and the more deeply located parts of the appendages are damaged to an equal extent and hence repopulation must occur from cells that have not been hit or from cells outside the radiation field. Finally heat produces no decrease in the ability of the endothelial cells of the blood vessels below the depth of the burn, to bud and proliferate. As a result there is not the reduction in the tissues' tolerance to trauma in thermal burns as is seen in irradiated skin.

The action of radiation on the proliferating cells of tumors will be our final example of this primary effect. Radiation is effective in cancer therapy not because the cells that make up tumors are particularly radiosensitive. We have seen that all cells have about the same sensitivity. Instead, the basis of radiotherapy depends on the fact that a tumor grows by cell multiplication. Those tumor cells that have been "hit" by radiation will, on attempting to divide, either differentiate, form giant cells or die. They will be unable to continue multiplying. The results that have been obtained with tissue culture experiments are extremely useful in understanding the radiation response of the population of cells that make up the tumor.

The dose of radiation required to assure that all the cells of a tumor have been "hit" and are incapable of further multiplication depends on the number of cells of which it is composed. The larger the tumor the larger the dose required. A small (2 x 2 x 2 cm3) tumor may consist of perhaps $10^{-10}$ cells. If we extrapolate the data of Fig. 1 to the right (to higher doses and lower



A B

Fig. 5. Normal rabbit skin (a) is compared with skin 15 days after an acute local dose of 5000 rads. The epidermis is reduced to a single layer of giant cells and there is a marked inflammatory response to the resulting cellular debris (H and E x 100).

survivals), we find that a dose above 3000 rads is necessary to "kill" all these cells. None of the irradiated cells will be able to multiply.

In many cases it is, however, impossible to irradiate all the tumor to this dose and not damage a vital organ at the same time. This is because beams of radiation scatter and the energy they contain may be absorbed by normal tissue in the surrounding region. But even if it were possible to deliver a very sizeable dose to a specified volume and no other, it would still be difficult to delineate the margins of the tumor exactly. If the field is increased in an attempt to catch any locally invading cells, the dose must be reduced because normal tissue is necessarily being irradiated simultaneously.

These fundamental limitations in the application of radiotherapy have attracted much attention. In order to assure a large dose to the tumor, high energy x-ray and gamma-ray machines have been invented and multiple field and rotational therapy has been developed. To ensure that the entire tumor is being irradiated, improved tumor localization has been implemented and experiments with *in vivo* isotope labelling of tumors initiated.

While many of the phenomena of tumor growth may be understood by comparing the tumor with a test tube of cells in suspension, this is certainly a simplification. The cells of the tumor depend on blood for their nutrition and as they increase in number, the vessels increase to supply it. Following radiation these vessels may be incapable of proliferation and the tumor so limited in its growth. The effectiveness of radiotherapy may be partially due to this action on the host's blood supply.

Then many experimental tumors and certainly some human tumors elicit an antibody response in the host. It is possible that after a large fraction of these cells are destroyed the antibody is more effective in destroying the remaining tumor. This may explain the rapid disappearance of some tumors after a small dose of radiation.

Finally, a dose to a tumor sufficient to block all the cells from proliferating does not insure that the tumor will not grow in size. Many of the irradiated cells may become hyperploid giant cells and the tumor expand and kill despite the fact that it is composed of cells that are incapable of continued division.

## RADIATION-INDUCED MUTATIONS

At radiation dosages insufficient to prevent proliferation of cells, there may still be sufficient damage to the macromolecular structure of the genetic apparatus so that mutations are produced. Briefly we may say that there may be two types of mutations: genetic mutations which occur in germ cells and somatic mutations which occur elsewhere in the body.

If a germ cell which has suffered a genetic mutation is fertilized, the product so formed carries it but the mutation may not be expressed for generations. In the vast majority of cases, these mutations are detrimental so that some estimate of the dose required to produce such effects is of great importance.

The radiation dose that we all receive in a year from natural sources (cosmic rays and natural radioisotopes) is about 0.1 rads per

year. From birth to reproduction at 30 years of age, each individual then receives on the average 3 rads (11). This continued exposure of our population to natural, not man-made, radiation results in a small fraction of all births exhibiting hereditary defects. It is clear, therefore, that any unnecessary exposure of the population is bad and that the benefits of the use of radiation must always be weighed against this possible harm. In many procedures the gonadal dose is so small that it hardly needs to be considered. A miniature chest x-ray taken with properly installed equipment, for instance, gives a gonadal dose of less than 0.0003 rads. But x-ray examinations of the pelvis may yield sizeable gonadal doses and the hazard of producing genetic mutations should be borne in mind.

Somatic mutations form a hazard to the individual who suffers them rather than to succeeding generations. This is because any somatic cell may suffer a mutation to a form that is no longer sensitive to the controls normally governing all multiplication and the cells may give rise to tumors.

The maligancy develops in the area irradiated. For instance, prolonged exposure of the hands to radiation may be followed by squamous cell carcinoma in the exposed areas. Ingested radium is deposited in bone and may be followed by osteogenic sarcoma. Radioactive dust particles such as may be inhaled by those who mine radioactive ores are trapped in the lung and may give rise to malignancies there.

The incidence of malignancy increases with the dose of radiation but with modern industrial hygiene, where radiation monitoring strictly controls the exposure of personnel, there is little opportunity for an individual to receive a dose sufficient to induce a malignancy (12).

## CYTOPLASMIC DAMAGE TO CELLS

In contrast to the nucleus, the cytoplasm of a cell is very insensitive to radiation. It is for this reason that even moderate doses of radiation have their most prominent action on cells in division. But very large doses may reduce the function of all cells and destroy many by cytoplasmic damage. Situations where this is important are uncommon though nervous system death following a massive dose of radiation is probably of this origin.

The mature lymphocyte is the one exception to the general insensitivity of mature non-proliferating cells to radiation. The number of lymphocytes in the blood stream, lymph nodes and thymus decrease rapidly following whole body irradiation. This is perhaps not too remarkable as disintegration appears to be the lymphocyte fate and this is hastened by other agents such as cortisone and colchicine.

## ACUTE WHOLE BODY IRRADIATION

As an example of the principles we have elaborated, let us consider the effects of a single dose of radiation to the whole body. The pathological picture varies enormously with the dose but if we remember that the basic primary actions of radiations apply at each dose, we will realize it is only the relative importance of each that varies.

A very large dose of total body radiation, perhaps 10,000 rads in man, leads to death in a few hours. Somatic and genetic mutations occur but they will have little opportunity for manifestation. Nearly all the stem cells of the bone marrow and the germinal cells of the intestine are unable to proliferate but the mature

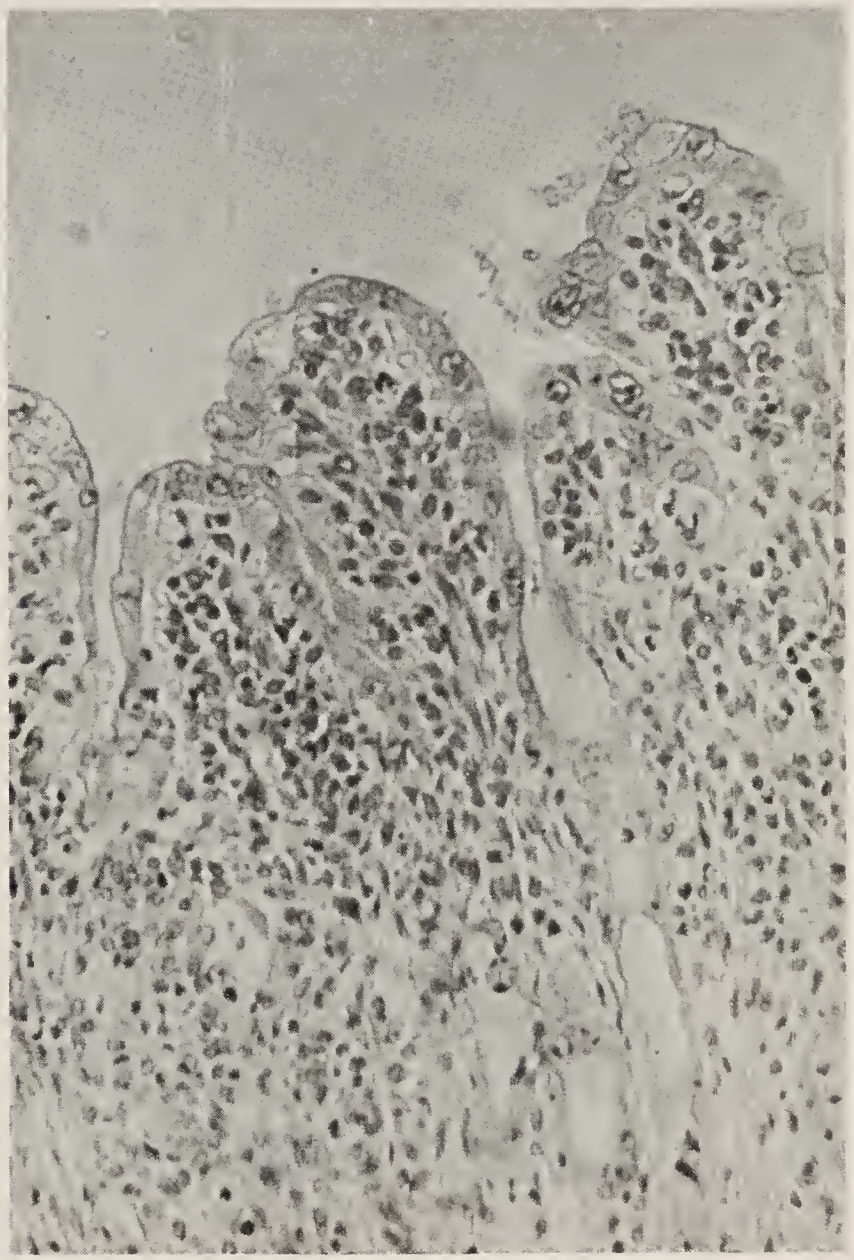


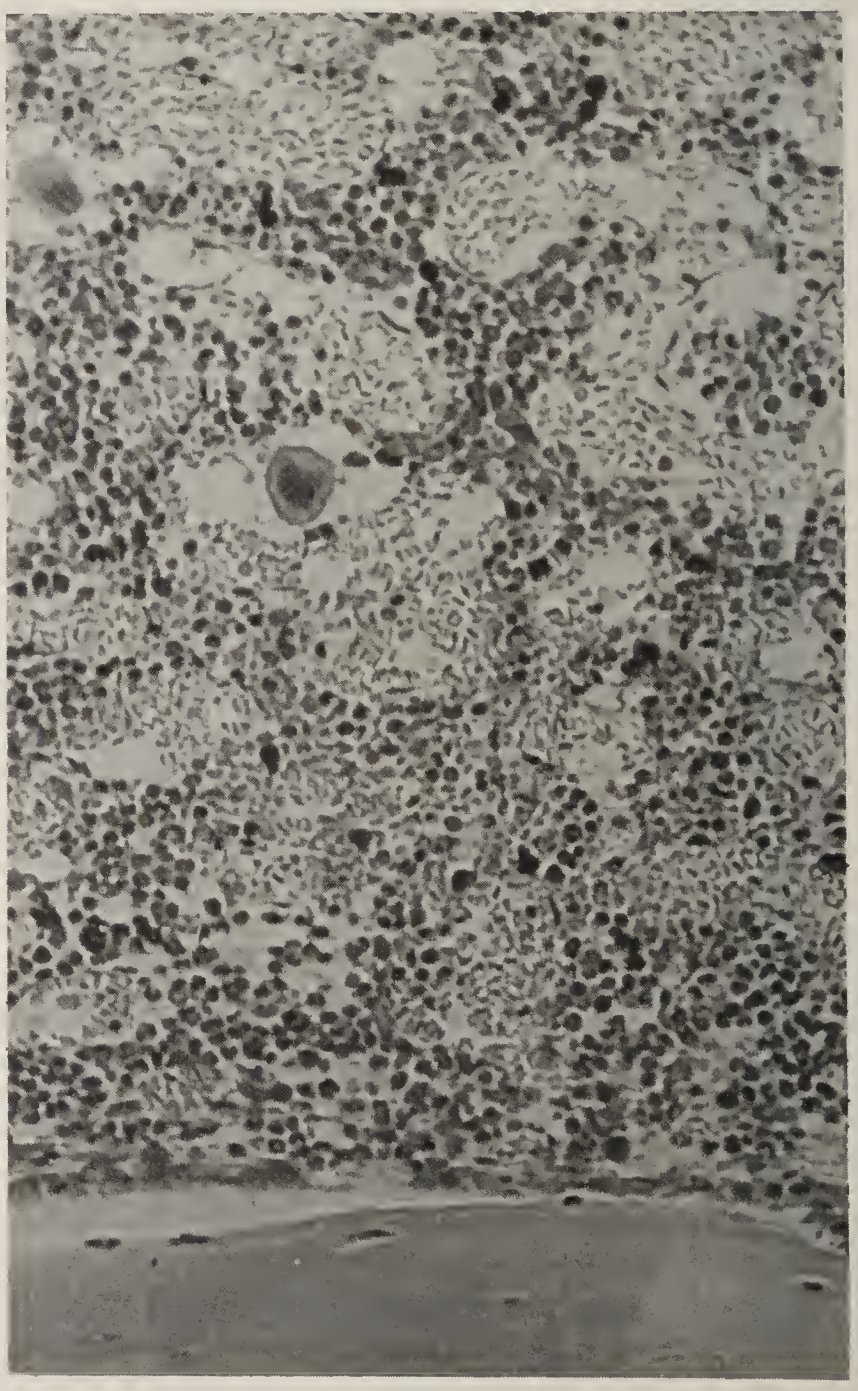
Fig. 6. The intestine of a rat 3 days following 1500 rads total body irradiation. Note the abnormal epithelical cells and the degree of infiltration. (H and E x 100).

cells already present at the time of irradiation keep these systems temporarily intact. Death is due instead to cytoplasmic damage of the non-dividing cells of the nervous system. Signs and symptoms are those of collapse and shock.

At lower doses of about 1500 r, this nervous system damage may be insufficient to cause death. The animal survives until, because of the block in cell multiplication, the epithelium of the gut becomes defective, ulcerated and denuded. The signs and symptoms may be due to electrolyte imbalance, water depletion or bacteremia which follows the gastro-intestinal ulcerations. A section of rat intestine following a total body dose of 1500 rads is shown in Fig. 6. The epithelial cells are abnormal in some areas and absent in others. Their effectiveness as an absorbing surface and bacterial barrier is understandably reduced. Bone marrow cells cannot proliferate effectively but the animal succumbs before leukocytes, platelets and red blood cells are depleted.

At doses from 250 - 900 rads, the gut is able to repopulate and survive because sufficient germinal cells escape being "hit". In a period of weeks, however, the peripheral blood counts are very abnormal. There is an initial rapid drop in lymphocytes, than a drop in the granulocytes. Then platelets and red cells may reach very low levels. These are due to the block in the proliferation of stem cells and a resulting depletion of maturing marrow cells as seen in Fig. 7. Death may be due to hemorrhage which is a result of platelet deficiency, or to infection, a consequence of the leukopenia and the di-

A



B

Fig. 7. Bone marrow of the normal rat (a) is compared to that of one 4 days following 450 rads total body irradiation. Note the absence of stem cells, the erythroid and myeloid series. Megakaryocytes persist at this time but much of the remainder of the space is occupied with blood filled sinusoids.

minished ability of the immunological apparatus to respond to antigens.

At dosages of radiation below the lethal dose, damage to the cytoplasm of cells is unimportant. Many of the proliferating cells are blocked from dividing but others that have not been "hit" by the radiation are able to repopulate the organ. The most important injuries are due to mutations. The life expectancy following total body irradiation with sizeable "sublethal" doses is definitely reduced. A sizeable part of this may be attributed to the high incidence of leukemia.

While it is not possible at present to affect the ultimate survival of animals that have received doses of radiation sufficient to cause "nervous system" or "gastrointestinal" death, it is possible to prevent the death of animals that

Fig. 8. The spleen of a mouse that received lethal total body irradiation and was subsequently treated with bone marrow. The nodules are clones arising from transfused stem cells.

have received sufficient radiation to cause "bone marrow" death. To do so viable bone marrow cells must be given intravenously following radiation. Some of the transplanted cells are stem cells and they, if given in sufficient numbers, are capable of repopulating the hematopoietic system and thus preventing a fatal leukopenia and thrombocytopenia.

It has recently been found that the repopulation occurs in the form of little clones of hematopoietic cells. Fig. 8 shows the spleen of a mouse that received 950 rads 10 days previously and 6 x $10^4$ bone marrow cells shortly afterwards. The nodules on its surface are found on microscopic examination to be clones of cells that have originated from a single transfused unirradiated stem cell. The quantitative possibilities that cell clone counting *in vivo* permits suggests many possible experiments with transplanted bone marrow.

Bone marrow transplantation studies with mice may make use of isologous animals that have antigenically similar cells. In humans this is seldom the case save when the donor and receptor are identical twins. In all other cases there will be differences between the antigens of the host and donor tissues. It now appears likely that the transplanted cells repopulate the immunological apparatus of the irradiated animal and produce antibody active against the host. The result, referred to as secondary disease, is typified by epilation in man.

## SUMMARY

We have attempted to give a concise picture of the response of an animal to radiation in terms of the consequences of three primary radiation injuries. These are mutations, the inhibition of cellular proliferation and the direct damage to the cytoplasm of cells. Of these three the second plays the largest role in the lesions of radiation pathology. One might expect then that our understanding of the effects of radiation on the intact animal will closely parallel advances in the study of the mechanism, control and rate of cellular proliferation generally.

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# BOOK REVIEWS

The Care of the Geriatric Patient: edit. E.V. Cowdry; C.V. Mosby Co., 1958, $ 8.00.

A few years ago Drs. Morris Piersol and Edwin Bartz summed up the concept of geriatrics with the following words: "The society which fosters research to save human life cannot escape the responsibility for life thus extended. It is for science not only to add years to life, but more important, to add life to years". To the fulfillment of that dictum this book is devoted.

The eminent contributors to this book have assembled comprehensive information about the treatment of geriatric patients from the physical to emotional aspects of their care. Prevalent theories of aging and biologic factors involved in this phase of life are discussed along with the tolerance and altered reactivity of the aged to widely used drugs. The attention of the reader is focussed on the psychiatric complaints of old people who often develop multiple signs and symptoms simulating organic disease to attract the attention of those around them.

Medical rehabilitation for this age group is suggested to be a control of diet and performance of physical exercise. It is realized that asymptomatic disease can often present itself; to help in diagnosis, the main pathological processes in the aged - metabolic deficiencies, loss of endocrine function, degenerative and malignant disease - are discussed fully. Finally, to complete the treatment, the standards of care expected and needed by the patient as well as the type of home required, whether nursing or private, are set forth.

The extent to which the practical and comprehensive steps in care recommended in this book are applied will decide whether the geriatric patient will spend his declining years as an institutionalized vegetable or whether his disabling condition will have been delayed long enough to enable him to lead an independant, or even partially independant, fruitful life.

A.H.G.

Medicine as an Art and as a Science: A.E. Clark-Kennedy and C. W. Bartley; J. B. Lippincott Co., 425 pages.

This delightful text manages to discuss most of the medicine which an undergraduate is required to know. As such, it is a must for those students who practise "medical upsmanship". In a simple, clear and logical language, the authors have provided a framework of basic knowledge on which the student can build his medical experience.

The text is divided into 5 parts; the patient and his disease, primary functional disorders, organic disease, clinical diagnosis, and the principles of prevention and treatment.

For those going into the second medical year, the information obtained from this book would be invaluable in imparting an overall picture of an introduction to medicine. For those in subsequent years, the text would serve adequately as a swift, yet effective means of recalling the pertinent facts of the clinical approach.

R.B.

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ABSTRACT: The Stimulus to Collateral Circulation: *H.T. John* and *R. Warren;* Surgery, Vol. 49 Jan. 1961, pp. 14-25.

Intra-arterial pressure tracings, arteriography, and venous occlusion plethysmography in dogs show that the stimulus to the development of collateral circulation around a block appears to depend on the extent of the pressure gradient between proximal and distal sides of the block. This leads to increased flow and dilatation of the collaterals, the pressure distal to the block rising over a period of hours to days until the pre-occlusion diastolic pressure is reached. Active exercise causes a fall in pressure distal to the block, while immobilization or perfusion of the distal circulation with oxygenated and hypoxic blood cause diminution of collateral channels.

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# BOOK REVIEWS

A Synopsis of Contemporary Psychiatry; G.A. Ulett and D.W. Goodrich: C.V. Mosby Co., 1960, 297 pages, $ 6.50.

This second edition of a text written, as usual, to serve "as a quick reference for psychiatric residents, medical and psychological interns, medical students, nurses, and others whose work in the psychiatric clinic and hospital may be for a brief period of time", is said to be published because the first edition found "sufficient usefulness to warrant a revision". Perhaps the usefulness was revised out of the second edition.

The text is poorly written and, like many synopses, is so packed as to obviate comprehension. As a reference book it is totally inadequate for it will not amplify what the student may already know nor does it make understandable definitions ("Phobic neurosis (anxiety hysteria) is a concept initiated by Freud to refer to cases where the anxiety is connected with a particular object or situation which symbolically represents the neurotic conflict"). An up-to-date series of charts at the end of the book would be a handy manual of drugs used in psychiatry were it not for the fact that the book is a little oversize for convenient pocket carriage. This synopsis might very well discourage the student's intrigue with psychiatry and is not recommended for any of those people listed above.

L.E.S.

Management of Fractures, Dislocations, and Sprains; J.A. Key, H.E. Conwell, and F.C. Reynolds: C.V. Mosby Co., Illus., 1129 pages, $ 27.00.

This seventh edition of a standard text sees a thorough revision of the former editions originally written by the late Dr. Key and Dr. Conwell. Methods of treatment now obsolete have been deleted and replaced with new material. Deceleration injuries and spinal disc involvment have been given more detailed discussion. Treatment by medullary nail and hip prosthesis is discussed. Special chapters on anesthesia and injuries of the hand have been included and revised, respectively.

Although too large for complete reading at this time of year, this book, as sampled, was found to be of excellent quality. The numerous illustrations and radiographs are an exceptional complement to the text. If unable to go the prohibitive $ 27.00 while in medical school, do not forget this valuable reference when you begin practice.

L.E.S.

Atlas of Obstetric Technic; J.R. Willson: C.V. Mosby Co., Illus., 301 pages, Deluxe Edition - $ 14.50, Regular - $12.50.

Although the author states that this atlas is not to serve as a basic text for medical students, it would be a bonus to them to have a copy. Obstetric technic, bandied about in the lecture theatre and consequently ill-understood, comes to life in the pages of this book.

The illustrations as done by Miss Daisy Stillwell are so self-explanatory that little text is needed and, in fact, little is used. Short notes tersely telling of the important steps in the procedures accompany the simple, clear drawings. The coverage is complete for all obstetric operations, normal delivery, forceps delivery, and abnormal presentations. An excellent production and a useful textbook, this atlas is well worth what, for medical texts, is a moderate price. As we received the DeLuxe edition for review, we cannot say how the regular edition differs except for the two dollars. L.E.S.

Importance of the Vitreous Body in Retina Surgery. Edited by Charles L. Schepens. 226 pp. illus. The C. V. Mosby Co. $ 15.00.

Light Coagulation. Gerd Meyer-Schwickerath. Translated by Stephen M. Drance. 114 pp. illus. The C. V. Mosby Co. $ 9.50.

The first of these books is a record of the second conference of the Retina Foundation, held in 1958. The chief problem dealt with at the conference was that of retinal detachment. The first part of the book deals with research into the basic physiology and pathology of this condition. Such possible etiological factors as cellular proliferation at the retinal-vitreous junction, and developmental defects are discussed. The last part is devoted to the newer techniques of treatment of retinal detachment.

The second volume is a monograph describing the author's experience in treating a wide variety of ocular conditions by means of light coagulation. This is a relatively new technique which utilizes a high energy light source to produce therapeutic burns in the retina. By varying the degree of exposure, effects ranging from a proliferative response, as in the treatment of macular holes, to destructive effects in the treatment of ocular neoplasms can be obtained.

Obviously the highly specialized nature of these books, to say nothing of their formidable price, limits their appeal to the specialist in this field, who no doubt will find in them new and valuable information.

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